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AND PARASITOLOGY**

THE UNIVERSITY OF LIVERPOOL

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PARASITOLOGY

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CONTENTS

No. 1. June 30, 1920

| | PAGE |
|---|------|
| MILNE, A. H. | |
| In Memoriam | 1 |
| GASKELL, J. F. | |
| Notes on Blackwater Fever in Macedonia | 3 |
| EDWARDS, F. W. | |
| The Nomenclature of the Parts of the Male Hypopygium of Diptera Nematocera, with Special Reference to Mosquitoes | 23 |
| SWELLENGREBEL, N. H. ; and SWELLENGREBEL DE GRAAF, J. M. H. | |
| Studies on the Various Types of Malarial Infection and the Effect of Quinine Treatment thereon among the Native Population of the Malay Archipelago | 41 |
| MACFIE, J. W. S. | |
| Heat and <i>Stegomyia fasciata</i> : Short Exposures to Raised Temperatures .. | 73 |
| MACFIE, J. W. S. ; and FRASER, M. W. | |
| I.—Oral Administration of Quinine or Quinine and Arsenic for Short Periods to Young Native Children Infected with Malignant Tertian Malaria ... | 83 |
| MACFIE, J. W. S. | |
| II.—Oral Administration of Quinine Sulphate Grains 20 to Adult Natives Infected with Malignant Tertian Malaria | 93 |
| MACFIE, J. W. S. | |
| III.—Oral Administration of Quinine Sulphate Grains 10 Daily for Two Consecutive Days only to Native School-Boys Infected with Malignant Tertian Malaria | 95 |
| MACFIE, J. W. S. | |
| IV.—Oral Administration of Quinine Sulphate to Natives Infected with Quartan and Simple Tertian Malaria | 111 |
| BLACKLOCK, B. ; and CARTER, HENRY F. | |
| On the Results Obtained from Surveys for Breeding-Places of Tree-Hole Mosquitoes in Liverpool and Neighbourhood | 115 |
| YORKE, WARRINGTON ; and SOUTHWELL, T. | |
| <i>Crossocephalus zebrae</i> , n.sp. | 127 |

CONTENTS

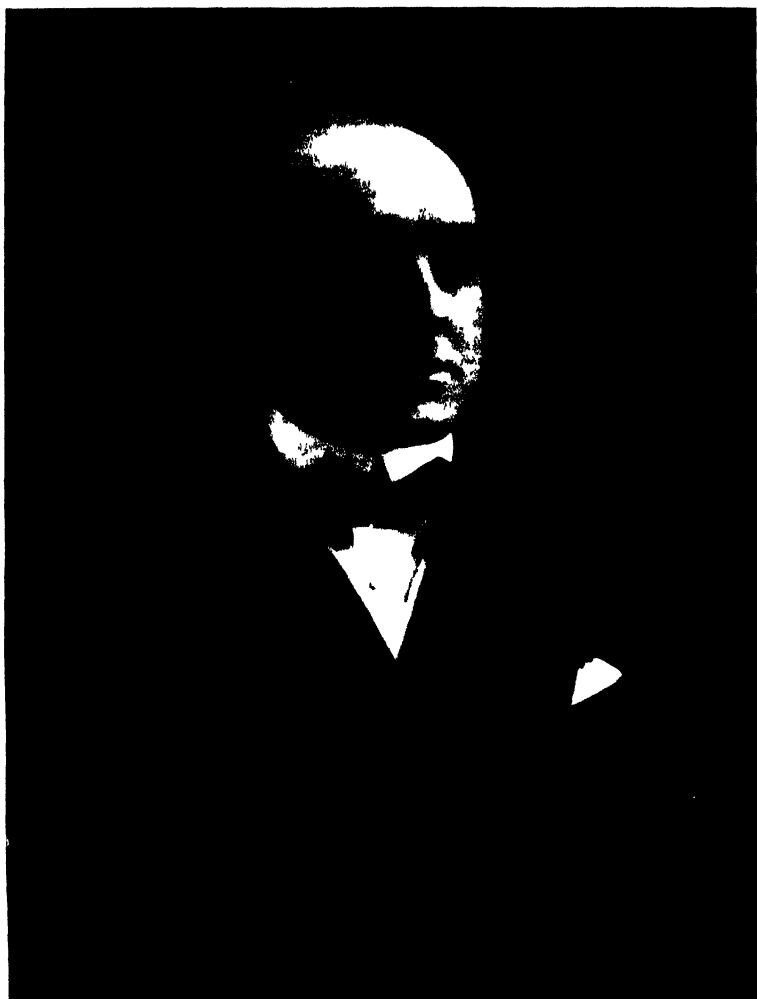
No. 2. November 27, 1920

| | PAGE |
|--|------|
| MACFIE, J. W. S. | |
| Tartar Emetic in Guinea-worm Infections | 137 |
| MACFIE, J. W. S.; and INGRAM, A. | |
| Three Cases of Cardiac Aneurysm in Native Boys of the Gold Coast | 147 |
| YORKE, WARRINGTON; and MACFIE, J. W. S. | |
| <i>Strongylidae</i> in Horses: IX.— <i>Cylicostomum tridentatum</i> , sp.n. | 153 |
| YORKE, WARRINGTON; and MACFIE, J. W. S. | |
| <i>Strongylidae</i> in Horses: X.—On the Genus <i>Poteriostomum</i> , Quiel | 159 |
| YORKE, WARRINGTON; and MACFIE, J. W. S. | |
| <i>Strongylidae</i> in Horses: XI.—Species found in West Africa and Jamaica ... | 165 |
| YORKE, WARRINGTON; and MACFIE, J. W. S. | |
| <i>Strongylidae</i> in Horses: XII.— <i>Cylindropharynx rhodesiensis</i> , sp.n. | 169 |
| YORKE, WARRINGTON; and MACFIE, J. W. S. | |
| <i>Strongylidae</i> in Horses: XIII.— <i>Cylicostomum triramum</i> , sp.n. | 175 |
| SOUTHWELL, T. | |
| Fish and Mosquito Larvae in Bengal, Bihar and Orissa, India | 181 |
| CARTER, HENRY F.; INGRAM, A.; and MACFIE, J. W. S. | |
| Observations on the Ceratopogonine Midges of the Gold Coast with Descriptions of New Species. Part I | 187 |
| CARTER, HENRY F.; INGRAM, A.; and MACFIE, J. W. S. | |
| Observations on the Ceratopogonine Midges of the Gold Coast with Descriptions of New Species. Part II | 211 |
| BLACKLOCK, B.; and CARTER, HENRY F. | |
| Further Experiments with <i>Anopheles plumbeus</i> , Stephens; Its Infection with <i>P. falciparum</i> in England; also Notes on the Apparatus and Technique Employed | 275 |

CONTENTS

No. 3. February 8, 1921

| | PAGE |
|--|------|
| MAPLESTONE, P. A. | |
| Human Intestinal Protozoa in North Queensland | 283 |
| SOUTHWELL, T. | |
| A Note on the Occurrence of certain Cestodes in New Hosts | 295 |
| KOTLÁN, ALEXANDER. | |
| Two New <i>Cylicostomum</i> Species from the Horse | 299 |
| CARTER, HENRY F.; INGRAM, A.; and MACFIE, J. W. S. | |
| Observations on the Ceratopogonine Midges of the Gold Coast with Descriptions of New Species. Part III | 309 |
| BEZZI, M. | |
| <i>Musca inferior</i> , Stein, Type of a New Genus of Philaematomyine Flies (<i>Diptera</i>) | 333 |
| STEPHENS, J. W. W. | |
| Some Statistics of Filariasis | 341 |
| SOUTHWELL, T. | |
| A New Species of Cestode (<i>Anoplocephala vulgaris</i>) from an African Rhinoceros | 355 |
| STEPHENS, J. W. W.; YORKE, W.; BLACKLOCK, B.; and MACFIE, J. W. S. | |
| Studies in the Treatment of Malaria: XXXI.—The Time of Onset of the Paroxysms in Simple Tertian Malaria | 365 |
| STEPHENS, J. W. W.; YORKE, W.; BLACKLOCK, B.; MACFIE, J. W. S.; COOPER, C. FORSTER; and CARTER, H. F. | |
| Have Differential Leucocyte Counts any Value?... .. | 371 |
| BREINL, A. | |
| Preliminary Note on the Development of the Larvae of <i>Dirofilaria immitis</i> in Dog Fleas <i>Ctenocephalus felis</i> and <i>canis</i> | 389 |



A. H. Milne.

ERRATA

Vol. XIII, p. 304, line 18, for 'ten days, no interval greater than ten days' read 'X days, no interval greater than X days.'

p 305, line 1, for 'within our ten days,' read 'within our X days.'

In Memoriam

ALLAN HAY MILNE, C.M.G.

Allan Hay Milne, born in 1869, was the youngest son of the late Very Reverend A. J. Milne, LL D., of Fyvie, Moderator of the General Assembly of the Church of Scotland. He was educated at Fettes College, Edinburgh, and Pembroke College, Cambridge. He entered the service of the Liverpool Chamber of Commerce in 1895, and was promoted to the Secretaryship in 1913. In 1911 he was made a Companion of the Order of St. Michael and St. George. He became Honorary Secretary of the School of Tropical Medicine on February 7th, 1899, and resigned his post through ill-health in October, 1917. He always devoted himself with conspicuous energy and success to the interests of the School, and his death on 21st January, 1918, left a vacancy which it will be difficult to fill. His bright, genial temperament and his intellectual attainments were always an attractive stimulus to those with whom he was associated.

We desire to record here our appreciation of these services, and to express our sense of the loss we have experienced.

NOTES ON BLACKWATER FEVER IN MACEDONIA

BY

J. F. GASKELL

From the 41st General Hospital, Salonika

(Received for publication 11 March, 1920)

I. INTRODUCTION

The nature of blackwater fever and its relationship to malaria and to the administration of quinine are questions at present undecided. The extensive work of Christophers and Bentley in India (1908), of Stephens (1903) in Africa and elsewhere, and especially the exhaustive investigations of Deeks and James (1911) during the building of the Panama Canal, furnish very strong evidence that blackwater fever is intimately related to malaria. They conclude that it only occurs when a population non-immune to malaria becomes exposed to extensive infection by it, when relapse and reinfection are therefore common, and owing to unfavourable conditions proper quinine treatment has not always been obtainable. Their observations also show that quinine has in a very high percentage of cases been the determining cause of the attack: where attacks have occurred when the patients have been under observation in hospital, the antecedent administration of quinine has been practically universal. The conditions quoted above were all present among the allied forces in the Balkan campaign of 1915 to 1918, and blackwater fever duly appeared. Conditions were especially favourable in the Serbian army, for not only was malaria a comparatively rare disease in Serbia, but also the army became highly infected when training at Salonika in 1916, and owing to various causes proper treatment often could not be given.

During the autumn and winter of 1917-1918, seven cases of the disease came under observation in the 41st General Hospital at Salonika. As they demonstrate certain points having bearing upon the above questions, I have thought it worth while to record them,

more especially as the observations were made when completely ignorant of the literature of the disease, and thus afford independent confirmation of the views given above. Certain cases were under continuous observation in hospital for a considerable period, and suffered from repeated attacks of both malaria and blackwater fever, which could therefore be studied from their earliest stages. The 41st General Hospital acted as Base Hospital to the Serbian army from August, 1916, onwards, and six of the patients who suffered from blackwater fever were Serbians, the remaining case belonging to the hospital personnel. A further series of forty-six cases, mostly Serbians, have been recorded by Parsons and Forbes (1919), who state that their cases varied considerably in severity, but conclude that they were dealing throughout with a single disease, and that a separation into quinine haemoglobinuria and blackwater fever was not justifiable. The seven cases, with which this paper will deal, also varied considerably in severity, but were essentially of the same nature. Twelve attacks of blackwater fever occurred among these seven cases, eight taking place in the hospital itself and following rapidly upon the administration of quinine. The severity of the illness differed according to the nature of the treatment given. Even if some of the attacks here recorded can be considered examples of quinine haemoglobinuria, the fatal attacks, such as those of Cases 1 and 3, were also directly determined by the administration of quinine, so that quinine caused both blackwater fever and quinine haemoglobinuria.

The distinction between the two conditions would then merely lie in an arbitrary standard of severity of the attack, a severity which can undoubtedly be influenced by the treatment adopted. The assumption of a distinct condition of quinine haemoglobinuria is on the evidence of these cases a doubtful one, and the milder cases are rather to be looked upon, in accordance with the conclusions of Parsons and Forbes, as mild cases of blackwater fever. That such mild cases may finally die from a very severe attack is shown by a case recorded by Koch (1899). The patient, a sufferer from benign tertian fever, had had about ten attacks of haemoglobinuria which always followed the taking of quinine. He died within twelve hours from a final attack of blackwater fever, caused by the subcutaneous injection of half a gramme of quinine.

II. THE RELATIONSHIP OF MALARIA TO BLACKWATER FEVER

By the beginning of August, 1916, when British Base Hospitals were first provided, the Serbian army had become widely infected with malaria; and the great majority of the patients admitted to the 41st General Hospital during this autumn were suffering from the disease, often in a very severe form. Nevertheless, during the autumn and winter of 1916-1917, though some thousands of cases of malaria passed through the hospital, no cases of blackwater fever occurred. With the exception of a few cases in the early spring of 1917, the disease did not make its appearance in the Serbian army till the autumn and winter of 1917-1918, when the army had passed through two severe malarial seasons, and the great majority of the Serbs were sufferers from chronic malaria. Evidence of chronic malaria was present in all the cases of the present series; in all the spleen was enlarged and palpable during life, and a history of previous malarial attacks was obtained in all but two.

The coincidence of blackwater fever and the establishment of chronic malaria in a community hitherto practically free from both diseases supports the view that the relationship between them is intimate. Further, the absence of blackwater fever from the Serbian army during the first autumn and winter of 1916-1917 is most remarkable if the disease is an independent one, having no relationship to malaria, for the ground occupied by the Serbian army was practically the same throughout the two winters, and the conditions of existence were greatly improved during the second period. Susceptibility to a new and independent disease should therefore have been greater during the first season of 1916-1917, yet blackwater fever did not then appear. If, however, blackwater fever can only occur in patients with chronic malaria, the absence of the disease in the first season is explained, for malaria patients were then not sufficiently chronic to be susceptible. This evidence is, therefore, in favour of the view that blackwater fever only occurs in patients with chronic malaria.

The severity of the malarial attacks and the nature of the infecting parasite, whether benign or malignant, does not appear to be of importance. The disease was certainly not confined to severe infections with the malignant parasite, as many writers have

suggested; but, as was found by Koch (1899) and Deeks and James (1911) in the Panama Canal Zone, it was associated with both the benign and malignant forms, though the latter authors conclude that the benign is of comparatively little importance. In four of the seven cases, benign tertian parasites were found associated with definite malarial attacks. They were identified in Cases 4, 5 and 6 at the onset of subsequent malarial relapses (Table II), and in Case 7 in the malarial attack occurring the day before the onset of blackwater fever. The relapse in Case 4, in which benign tertian parasites were found, was also followed by an attack of blackwater fever. In the remaining three cases no parasites were found, as films were not examined until after the onset of the fatal attack. No parasites have been found in this series when blackwater fever has become established. This should not, however, be taken to mean that blackwater fever has no relationship to malaria, but is rather to be interpreted in the light of cases, such as numbers 4 and 7 in this series, in which parasites were found immediately before the blackwater fever attack, but disappeared when the attack took place. The probable explanation of the absence of malarial parasites in the peripheral blood during an attack of blackwater fever is that the attack itself destroys them.

The degree of malarial cachexia present is obscured by the jaundice and anaemia due to blackwater fever itself, but in some of the cases of this series the cachexia was certainly not great, as they were not wasted to the extent which is then found. Patients suffering from a comparatively mild degree of chronic benign tertian malaria were, therefore, susceptible to blackwater fever as well as those with the more severe forms of malaria.

To sum up, blackwater fever in Macedonia was always associated with chronic malaria, which was not, however, necessarily of a severe type.

III. SEASONAL PREVALENCE

Six of the seven cases occurred between October, 1917, and April, 1918. Three of these had further attacks, which also took place within this period. This agrees with the seasonal incidence observed by Parsons and Forbes, the great majority of whose cases

occurred during the same period. The disease was, therefore, a winter one, and appeared when, owing to exposure to cold, malarial relapses were common, but fresh infection by malarial parasites did not take place. As the hospital consisted throughout its existence of tents only, patients could not be protected from the cold, and in consequence malarial relapses were frequent. The common history was a chill which brought on a malarial relapse, with which the attack of blackwater fever was associated. After April, 1918, no further cases of blackwater fever had occurred up to the end of August. The winter prevalence of the disease was thus very definite, and exposure to cold appeared in many cases to be a determining factor.

IV. THE RELATIONSHIP OF QUININE ADMINISTRATION TO THE ATTACK OF BLACKWATER FEVER

The particulars of the eight attacks of blackwater fever which occurred in the hospital are given in Table I. The time relationships of the doses of quinine given and the onset of the attack of blackwater fever are shown, as far as they could be ascertained, in the charts at the end of this paper. The attacks, with the exception of the third known attack of Case 2, all quickly followed the beginning of a course of quinine treatment in the hospital. The third attack of Case 2, which occurred six days after the second attack, took place while the patient was undergoing a full course of quinine, which consisted of 160 grains given intramuscularly spread over the first five days, and 30 grains per day orally on each of the following days. Quinine was therefore being administered in quantity when this attack took place. This third attack was so severe that the patient died before haemoglobin had appeared in the urine. It was substantiated by the discovery of haemoglobin in quantity in the kidney tubules after death.

Case 1 developed blackwater fever on the third day after admission, when he had undergone two days of the intramuscular course usually given. He gave a history of malarial attacks every other day for the ten days preceding admission, and stated that he had had quinine by mouth every day. The amount given must however have been small, as the malarial attacks were not controlled

and the patient's temperature was 100° on admission. The other attacks given in the table all rapidly followed the administration of quinine given on account of an attack of malaria. In one case only was the malarial attack doubtful, namely, in the second attack of Case 6. This patient was admitted suffering from his primary attack 14 days before, and, though this attack of blackwater fever passed off, his temperature did not fall to normal (Chart VI). Notwithstanding the fact that blood films were all found negative it was thought possible that the raised temperature was due to malignant malaria. A modified course of intramuscular quinine of 20 grains daily was decided upon, which brought on the second attack of blackwater fever on the third day. As subsequent malarial attacks proved that the case was one of benign tertian malaria, parasites should have been easy to find in the blood in any attack, but as the patient suffered from pyorrhoea, and an abscess subsequently declared itself at the site of a subcutaneous saline injection given shortly after admission, the temperature chart is probably explainable from the two last causes. The point is of interest because this attack would then be due to the administration of quinine only, and not to the combination of quinine and a fresh malarial attack. That is to say, an attack of blackwater fever can be brought about in a patient with chronic malaria by the administration of quinine, without the additional factor of an acute malarial attack. A similar case is recorded by Deeks and James (1911).

The initial attacks of Cases 2 and 4 occurred before admission to the hospital, and particulars regarding quinine administration were only vaguely obtained. Case 2 was admitted to a casualty clearing station on the third day of an attack of malaria and was given doses of 15 grains of quinine orally two days later, on which day blackwater fever declared itself. It is improbable that more than two doses, that is to say 30 grains, were given before the attack of blackwater fever. The initial attack of Case 4 occurred over a month before admission, and no details of quinine administration could be obtained. That quinine had relationship to the attack is, however, almost certain, as the patient stated that no quinine had been given him in the hospital from which he was transferred, and, though put on quinine on admission, he refused to take it for three days. He had presumably been instructed to this effect in

his previous hospital, but a satisfactory history was extremely difficult to obtain: it was only discovered that he had had an earlier attack after his second attack had declared itself. Cases 5 and 6 were admitted respectively on the second and third day of their primary attack of blackwater fever. Case 5 had been in our hospital six months before with malaria, and had had an illness with raised temperature for five days before the onset of blackwater fever. It is thus almost certain that he had taken quinine for this illness. Case 6 stated that he had been taking quinine irregularly for thirty days before blackwater fever began. There is thus presumptive evidence that these four initial attacks were related to the administration of quinine. In no less than three of these four cases subsequent administration of quinine rapidly produced further attacks, which gives additional support to the view that the primary attacks were caused in the same way.

The study of these seven cases thus shews an extremely close relationship between the administration of quinine and an attack of blackwater fever. All were cases of chronic malaria in whom relapses occurred, with only one exception, during the winter months; in all quinine was the final factor which determined the attack.

V. THE CRITICAL DOSE. QUININE TOLERANCE

The method of quinine administration in use throughout the hospital consisted of either 30 grains daily by mouth or 20 grains intramuscularly every morning and evening. The amount of quinine administered either by mouth or by the intramuscular method before the attack of blackwater fever is shewn in each case, where it was accurately known, in the last column of Table I, and varies from a single dose to a number of doses spread over two or three days. The amount necessary to produce blackwater fever was therefore different in each case, varying from 20 grains in Case 2 to 80 grains in Case 1. This variation gave rise to the idea that each patient susceptible to blackwater fever had a critical dose which produced the disease, and that smaller doses could be tolerated by him without ill-effect. This view has been already advanced by

Ziemann (1906) and Nocht (1905); the latter also maintains that tolerance can be built up by judicious quinine treatment. Cases 1, 2, 3 and 7 never became convalescent, so that the point could not be tested, but Cases 4, 5 and 6 all recovered and had subsequent malarial attacks, some of which were successfully treated by quinine without the recurrence of blackwater fever. The details of these are given in Table II:

It will be seen from Table I that Case 4 was unable to tolerate a dose of 30 grains without getting blackwater fever, whether it was administered orally or intramuscularly, but it will be seen from Table II that two subsequent attacks of malaria were, however, successfully treated by giving 10 grains a day, in the first instance intramuscularly, in the second, orally. Case 5 had many attacks of malaria in the hospital, and was able to tolerate a dose of 20 grains a day. Case 6 got a second attack of blackwater fever after three days' administration of 20 grains intramuscularly, but a subsequent malarial attack was successfully treated by 10 grain doses. The tolerance to quinine, therefore, varied in the three cases, but doses were found which the patient could tolerate in each case.

VI. THE EFFECTS OF QUININE ADMINISTRATION WHEN BLACKWATER FEVER HAS BEGUN

A full course of quinine administration was continued in the first three cases till death took place. Some quinine was also given after the onset of blackwater fever, in Case 5 who was extremely ill, and in Case 7 who died. In the other two cases (4 and 6) quinine was stopped directly signs of blackwater fever appeared, and none of their attacks shewed dangerous symptoms, but were quickly recovered from. Case 2 lived sufficiently long for a full course of quinine to be given, and though suppression of urine did not take place, was extremely ill, lying after the second day in a drowsy, apathetic condition. A third attack of blackwater fever just one week after the second was immediately fatal.

Case 5 was given 20 grains of quinine intramuscularly for two days after his admission on the second day of an attack of black-

water fever. He suffered from marked suppression lasting about a fortnight, and his illness was very severe.

Case 7 was given 30 grains intramuscularly on the day following the onset of blackwater fever, which had not then been discovered. He rapidly developed suppression which became complete, and he died on the fifth day notwithstanding the copious administration of fluids by every possible means. From the evidence of these cases the continued administration of quinine would appear to be most dangerous.

VII. COURSE OF ILLNESS

The onset of the attack was in some instances marked by a rigor, and the second attack of Case 4 probably dated from a fainting fit at 10 p.m. on the evening of February 7th. In other instances, the exact time of onset could not be accurately ascertained. The first appearance of haemoglobin in the urine probably occurs at a varying period after haemolysis has taken place, and is, therefore, not an accurate measure of the onset. In the final attack of Case 2 haemoglobin had not even reached the bladder when death took place, but was still confined to the kidney tubules. In some of the attacks here recorded, the first specimen of urine containing haemoglobin was the first passed that day, so that the true beginning of the haemolysis was even more vague, for it might have occurred at any time during the night. The onset of haemoglobinuria shewn by the thick line on the charts can, therefore, only be regarded as approximate. During the first day of the attack, even in the fatal cases, the general condition of the patient did not appear to be dangerous; for instance, Case 7, the hospital orderly, was under treatment for a malarial attack in his own tent, and did not feel sufficiently ill to ask for admission into a hospital ward until the second day of his illness. In the severe cases mental confusion and drowsiness became marked on the second day, often accompanied by an increase of the vomiting which had usually begun on the first day; vomiting was, however, completely absent in Case 2. A rapidly increasing jaundice was present in all cases, accompanied in the more severe by marked collapse with a feeble rapid pulse.

VIII. THE EXCRETION OF HAEMOGLOBIN AND OF ALBUMEN

The excretion of haemoglobin in the urine did not last many days in any attack, even when suppression was present. In the second and third attacks of Case 4, and the second attack of Case 6, which occurred in the hospital when these patients were being carefully watched and treated, haemoglobin disappeared from the urine within twenty-four hours. Even in Case 5, with marked suppression, haemoglobin disappeared four days from the onset; in the second attack of Case 2, where the general condition was very serious, although no suppression took place, the haemoglobin was all excreted within forty-eight hours. In this series, therefore, haemolysis, of which haemoglobinuria was a sequence, was complete within a comparatively short time, even though the clinical condition remained severe, and whether quinine was continued or not. Suppression became acute on the fourth day in Case 5, but, nevertheless, haemoglobin ceased to be excreted after that date. This shows that an almost complete loss of excretory power by the kidney does not cause excess of haemoglobin to be retained in solution in the blood. In Case 5, the last dose of quinine was given the day before haemoglobinuria ceased, but Case 2 shows that the cessation of quinine administration is not the cause of cessation of haemoglobin excretion, for here quinine was continued throughout, and yet haemoglobin disappeared from the urine in two days. In Case 2, however, the continued administration of quinine was accompanied by another attack of haemolysis seven days after the onset of the second attack. Haemolytic conditions had again been built up owing to the continuous supply of quinine to the blood.

The excretion of albumen was in every case continued after the excretion of haemoglobin had stopped. Its persistence was directly dependent upon the severity of the illness. In the second and third attacks of Case 4 it only lasted for four days, and in the second attack of Case 6 for seven days. In the more severe first attack of this case it lasted for twelve days. In the very severe attack of Case 5 with marked suppression it lasted for no less than sixty days.

IX. SUPPRESSION OF URINE AND THE CAUSE OF DEATH

Suppression was present in two cases, one of which was fatal. In both it reached its maximum on the fifth day, being complete in the fatal one, Case 7, while in the other, Case 5, the amount of urine was reduced to only $2\frac{1}{2}$ ounces.

Cases 1 and 3 died on the second day with a diminished output of urine. The third attack of Case 2 was so rapidly fatal that suppression could not have declared itself; the second attack of this case, though severe, was not accompanied by suppression.

Case 5 illustrates the time required for recovery from this dangerous complication; the process was gradual and the urine did not reach a normal amount for fourteen days, slowly increasing from its minimum on the fifth day. The condition of this case was still extremely critical for the fortnight following the recovery of full excretory power. Death in Cases 1, 2 and 3 was toxic in nature and too rapid for suppression to become manifest, though it would in all probability have occurred if the patients had lived longer, for the tubules of the kidney in Case 2 were distended with freshly excreted haemoglobin-stained material, and diminution of the amount of urine had already occurred in the other two cases. In Case 7 death rapidly followed the complete suppression of urine. Through the kindness of Captain Forbes, I was able to examine the kidneys of a number of other cases who had died with suppression of urine. The condition found was a complete obstruction of the kidney tubules, chiefly in the region of the loops of Henle, by plugs of coarsely granular material giving the haemoglobin reaction with eosin. The first convoluted tubules above the point of obstruction were dilated, but their epithelium was well preserved. The cause of suppression is thus a mechanical one. Warrington Yorke and Nauss (1911) have shewn that a similar condition can be produced experimentally by the injection of haemoglobin into the blood stream under conditions of diminished blood-pressure. The literature of the subject shews that suppression is the chief complication to be feared, if the toxic effects of the attack are not in themselves fatal.

With the exception of an extreme liquidity of the blood and the absence of malarial parasites and the changes in the kidney, the

fatal cases did not show post-mortem either macroscopically or microscopically any changes which are not met with in malaria. I have made an extensive series of investigations in malaria which are being published in the *Quarterly Journal of Medicine*, in which all the histological changes recorded by Whipple (1909) in blackwater fever have been found. The absence of malarial parasites in the tissues shows how complete is their destruction by the blackwater fever attack.

X. TREATMENT

In order to prevent the occurrence of suppression by the blocking of the kidney tubules, treatment should be directed to the administration of fluid in quantity, and at the same time collapse should be combated by the usual methods in conjunction with the administration of brandy and injections of strychnine. The experiments of Warrington Yorke and Nauss (1911) demonstrate the importance of the maintenance of blood pressure. In treating the earlier cases of this series the importance of the administration of fluids was not recognised. In Case 1, subcutaneous saline injections were only given very shortly before death, when the condition was desperate, and in Case 3 no extra fluids were given. Case 2 was given as much fluid as possible by mouth, but none by any other method. This treatment was begun in the early stages of his second attack, and possibly had considerable influence in preventing the occurrence of suppression. Case 5 was not given any great quantity of liquid until suppression had already set in on the fourth day of his illness; he was then, however, treated energetically both with rectal salines and as much fluid as possible by mouth, so long as suppression was still present. It is possible that in this case treatment by fluid in quantity begun on the day of admission might have averted the attack of suppression, but that the belated application of this treatment, nevertheless, enabled recovery to take place.

Cases 4 and 6 were treated from the earliest possible stage both with subcutaneous and rectal saline injections, and with as much fluid as possible by mouth. The importance of treatment by fluids, directly the first signs of blackwater fever occur, cannot be too greatly emphasised. This treatment should never be withheld

until signs of suppression appear; coagulation has then already taken place in the kidney tubules and the plugs cannot be dislodged. The object of fluid treatment is to dilute the excretions of the blood so as to prevent coagulation taking place.

XI. CONCLUSIONS

The details of the above cases support the view that the pathology of blackwater fever consists in the sudden occurrence of an extensive haemolysis in the blood stream brought about in certain cases of chronic malaria by the administration of quinine; exposure to cold is usually a contributory factor. The haemolysis is essentially chemical in nature and is completed within a comparatively short time, being in this respect very comparable to the haemolysis of paroxysmal haemoglobinuria. The excretion of the haemoglobin liberated can be successfully accomplished so long as it does not pass through the kidney in a too highly concentrated form. If it is too concentrated coagulation takes place in the loops of Henle, and suppression of urine occurs, which is usually fatal. Continuation of quinine administration increases the toxic conditions set up by the attack of blackwater fever and also increases the probability of suppression, though it does not appear to prolong greatly the period of actual haemolysis. Treatment should, therefore, be directed to the prevention of collapse and the dilution of both the haemoglobin and toxins by the administration of fluids in quantity by every available method. Quinine should be stopped immediately the blackwater fever is discovered; its further administration is both dangerous and also unnecessary, for the blackwater fever attack itself destroys the malarial parasites in the circulation.

In any particular patient a critical dose of quinine is necessary to produce an attack of blackwater fever; malarial attacks in such a patient can be successfully treated by doses below this limit.

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TABLE I.

Shewing the relationship of quinine administration to blackwater-fever.

| Case. | Attack of B.W. Fever. | Duration of malarial attacks before onset in days. | Number of days of quinine treatment preceding the attack. | Total amount of quinine in grains. | Remarks. |
|--------|-----------------------|--|---|------------------------------------|---|
| 1. ... | 1st | 13 | *10 ⁰ + 2 ¹ | 80 + | Amount orally in first ten days unknown, but certainly small. |
| 2. ... | 2nd 3rd | 9 15 | 1 ¹ 5 ¹ + 3 ⁰ | 20 250 | Quinine continued after attack. Haemoglobin in kidney only. |
| 3. ... | 1st | 2 | 1 ¹ | 40 | |
| 4. ... | 2nd 3rd | 2 2 | 1 ⁰ 1 ¹ | 30 30 | |
| 6. ... | 2nd | 0 | 3 ¹ | 60 | Malarial attack very doubtful. |
| 7. ... | 1st | 2 | 2 ⁰ | 50 | |

*10⁰ = ten days' oral treatment.2¹ = two days' intramuscular treatment.

TABLE II.
Critical Dose of Quinine.

| Case | Date of malarial attack. | Number of attack of Black-water fever. | Blood Film. | Dose in grains. | Number of days. | Total amount given. |
|------|--------------------------|--|-------------|--|-----------------|---------------------|
| 4. | 1918. Feb. 7 | 2nd | — | *Xo | 1 | 30 |
| | Feb. 26 | 3rd | B.T. | XX ⁱ (first dose) X ⁱ (subsequent dose.) | 2 | 50 |
| | April 2 | — | B.T. | X ⁱ | 6 | 60 |
| | April 25 | — | B.T. | Vo | 3 | 30 |
| 5. | Feb. 18 | — | B.T. | XX ⁱ (first dose.) X ⁱ (subsequent dose.) | 4½ | 90 |
| | Mar. 11 | — | Neg. | XX ⁱ | 3 | 60 |
| | April 1 | — | B.T. | XX ⁱ (first dose.) X ⁱ (subsequent dose.) | 3 | 60 |
| | April 5 | — | B.T. | XX ⁱ | 2 | 40 |
| | April 24 | — | B.T. | Xo | 3 | 60 |
| | | | | | | |
| 6. | Malaria doubtful. | 2nd | — | XX ⁱ | 3 | 60 |
| | April 25 | — | B.T. | X ⁱ (first dose) Vo (subsequent dose.) | 3 | 35 |

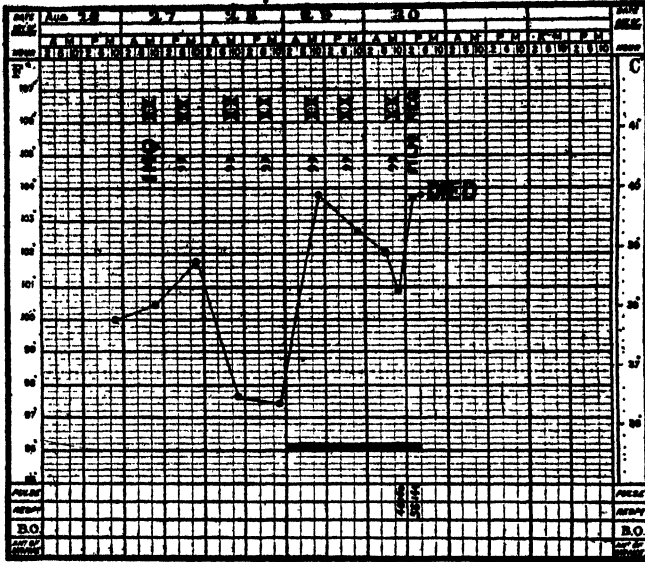
Xo = grains 10 orally.
XXⁱ = grains 20 intramuscularly.

EXPLANATION OF CHARTS

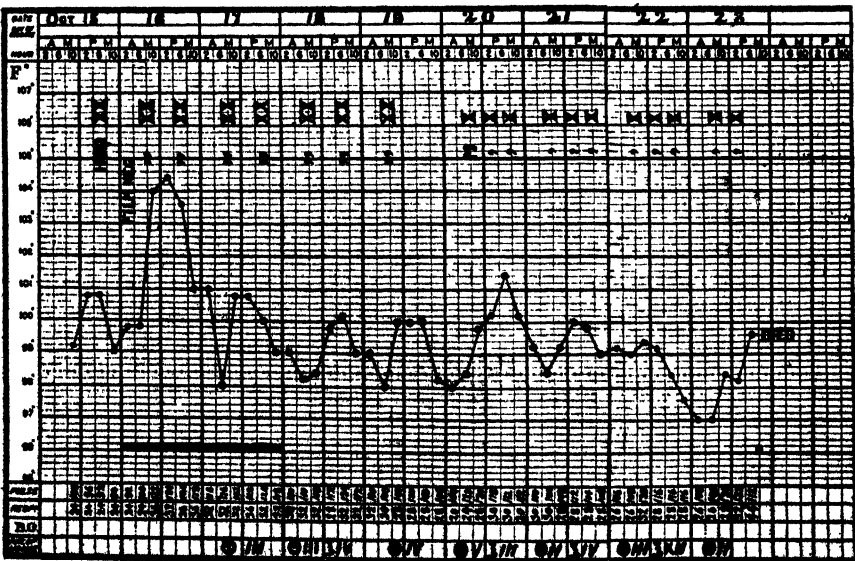
The thick black line shows the duration of the blackwater fever attack.

I.M.Q. = quinine intramuscularly.

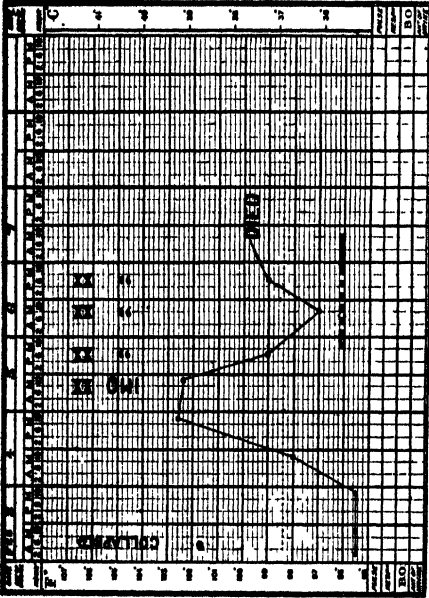
M. = quinine orally.



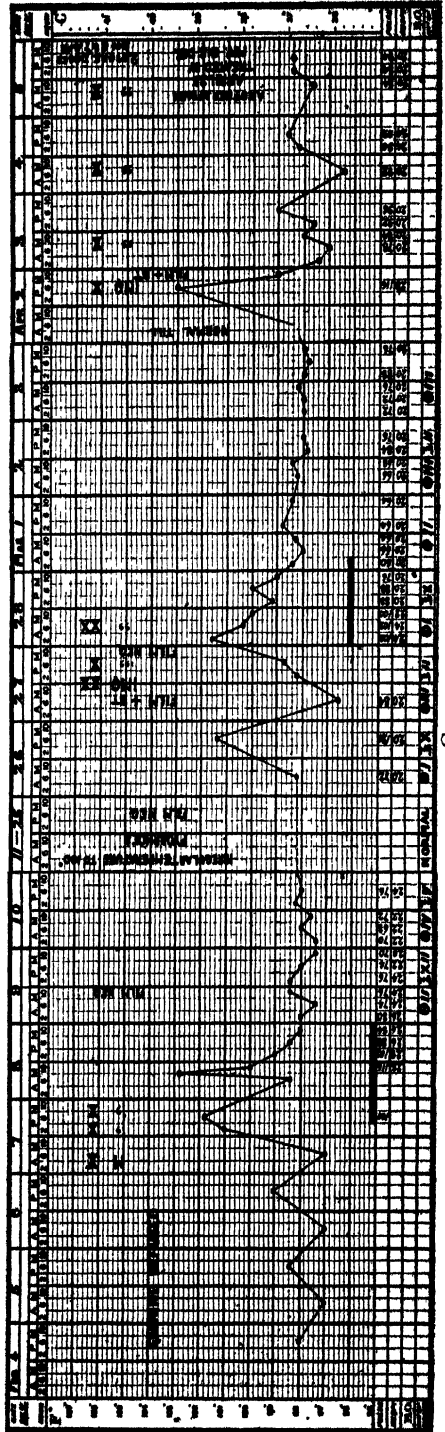
CASE 1



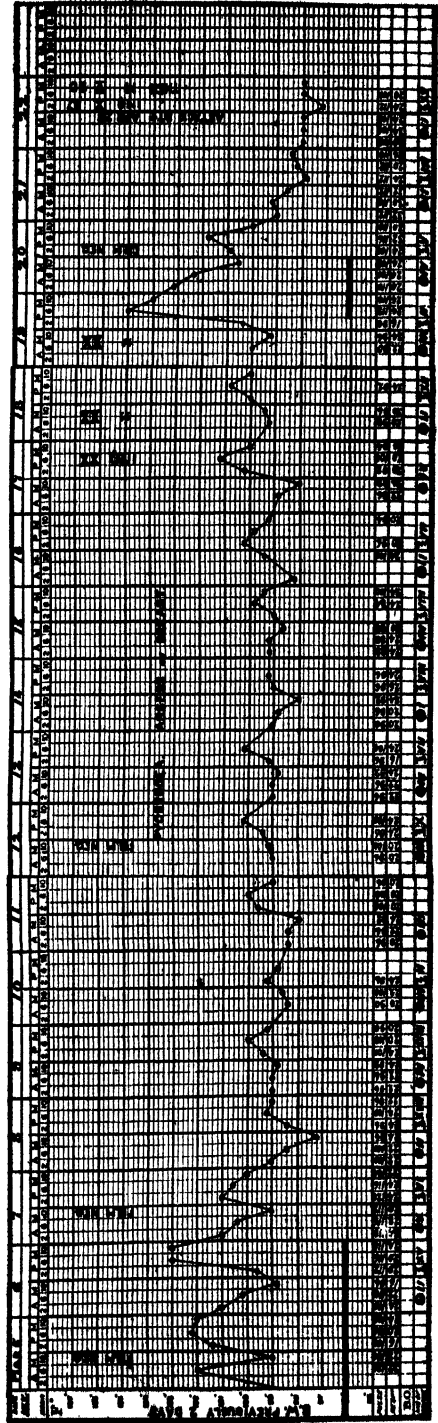
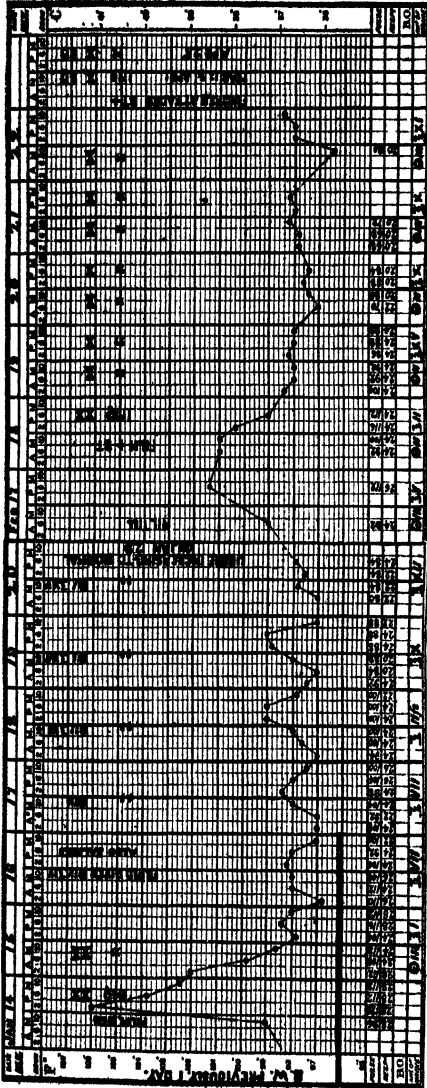
CASE 2



CASE 3



CASE 4



THE NOMENCLATURE OF THE PARTS OF THE MALE HYPOPYGIUM OF DIPTERA NEMATOCERA, WITH SPECIAL REFERENCE TO MOSQUITOES

BY

F. W. EDWARDS

(Received for publication 17 April, 1920)

It has long been recognised that the structure of the tip of the male abdomen is of the greatest importance in the classification of insects, and an enormous amount of work has been done in figuring the organs in different species. Unfortunately, however, the greatest diversity and confusion exists in the naming of the different parts, and nowhere is this more apparent than in the mosquitoes. The terms used for the lepidoptera have been applied to these insects in altogether different senses from those in which they were originally used, and, moreover, have been used even in different genera of the family Culicidae for structures which are clearly not homologous. The writer hopes in the notes which follow to indicate terms which can be generally adopted for all mosquitoes, and as far as possible those have been chosen which are applicable to other Diptera, if not also to other orders of insects. Since, however, the mosquitoes are amongst the most generalised Diptera in regard to genital structure, it must not be expected that the same terms will all apply to the highly specialised Brachycera and Cyclorrhapha. Reasonable allowance has been made for the claims of priority, but it has not been considered advisable to follow a rigid rule in this respect, and the name judged most suitable has been chosen in each case.

Of the many writers who have concerned themselves with the genitalia of mosquitoes, the only one who appears thoroughly to understand the subject is Christophers (1915). This author has given an accurate analysis of the parts in *Anopheles*, and has also pointed out the existence of a very remarkable and extremely

important phenomenon: that in *all male mosquitoes*,* *shortly after the emergence of the adult, the tip of the abdomen undergoes torsion through an angle of 180°*, so that the parts which are morphologically ventral become dorsal, and *vice versa*. It is probably failure to appreciate this fact that has led to most of the confusion in regard to the naming of parts; certainly such a failure has caused Brolemann (1919), the latest writer on the subject, to speak of the genital chitinisations as the 'cylindre perianal,' and the anal sclerites as the penis.

Good as Christophers' work was, it dealt only with *Anopheles*, where all the parts are not well developed, and so requires supplementing and slight modification.

Before proceeding further, it will be well to state the now universally accepted fact that the hypopygium of mosquitoes,† as of all but the more specialised metabolic insects, is composed of four distinct parts‡:—

- (1) A more or less continuous chitinous ring, representing the tergite and sternite of the ninth abdominal segment.
- (2) A pair of appendages of the ninth segment, more or less ventral in position (except secondarily in mosquitoes).
- (3) Chitinised pieces surrounding the anus.
- (4) Chitinisations of the genital tube, as opposed to the main body wall, which forms the other three parts.

We will discuss each of these elements in some detail.

(1) THE CHITINOUS RING OF THE NINTH SEGMENT. This is spoken of by Lepidopterists as the *tegumen*,§ the term having been introduced by Buchanan White (1878) and its use modified by P. H. Gosse (1883). Although in a great many insects, including

* Females are not so affected, but the phenomenon is not confined to the males of Culicidae. It occurs also in the males of Psychodidae (in *Plebotomus* and probably others) and Dixidae, and also in the Tipulid genera *Molophilus* and *Rhybolophus*.

† The present writer, in common with some others, has frequently spoken of the 'genitalia.' This term, however, should perhaps be confined to the chitinisation of the genital tube. *Hypopygium* is on the whole the best, used in the sense in which Snodgrass (1904) employed it, to include the four elements distinguished above; it is, however, not very appropriate for the Nematocera where the structures are terminal, not ventral as they are in Brachycera and Cyclorrhapha. Other terms in use by different writers are *male armature* and *copulatory apparatus*, but these seem less satisfactory.

‡ In a few cases the eighth abdominal sternite undergoes special development in the male, constituting a fifth element, but the occurrence is so rare that it may be dismissed with a mere mention. In any case there is no ambiguity about the term eighth sternite.

§ Not to be confused with the *tegmen* of the Coleopterata, which has an entirely different signification.

mosquitoes, it forms a continuous ring, yet this is not always the case, and even where it is, the ring is very much narrowed at the sides, leaving differential dorsal and ventral portions between which it is often possible to find traces of a suture. There seems, therefore, no need for the term *tegumen*, and it will be preferable to speak, as most writers on the hypopygium of Diptera now do, of the *ninth tergite* and *ninth sternite*.

The true ninth tergite is ventral in position in mosquitoes, as explained above. It is generally more or less distinctly bilobed, the lobes bearing bristles or spines, but its form varies greatly in different genera. I have used (1914) the morphologically incorrect term 'ninth sternite' for the whole structure; its lobes are the *setaceous lobes* of Felt (1905) and the *basal appendages* of Howard, Dyar and Knab (1912). This last term is inadmissible since the structures are not appendages. No objection can be raised to Felt's term, except that it is unnecessary. I propose to call these parts the *lobes of the ninth tergite*. The fact that they have sometimes been referred to as the lobes of the ninth *sternite* need cause no confusion, because the true ninth sternite is not usually lobed. These lobes have not been used as much as they might be in specific and even generic descriptions. Their most primitive form is seen in *Megarhinus regius*, where the tergite is broad, and without any emargination at the tip. In *Anopheles* and *Aëdomyia* the tergite is barely discernible and carries no bristles. In *Aedes* a pair of lobes are well developed, each nearly as long as broad, and bearing a row of spine-like bristles. The Sabethini show considerable specific differences, but the lobes are generally much more elongate than in *Aedes*, though sometimes shorter. In *Culex* they are broad and short and the terminal bristles are more hair-like. In some forms (e.g., *Uranotaenia pallidocephala*, *Armigeres obturbans*) the bristles are absent.

The ninth tergite is well developed and obviously distinct from the sternite in most families of Nematocerous Diptera, and often, particularly in the genus *Tipula*, exhibits most diverse specific modifications. In Cyclorrhapha, through the atrophy of the eighth or pregenital segment of Awati (1915) it is apparently the eighth: it is very large and folded back under the abdomen, covering the anal and genital parts. In *Phlebotomus* it is almost completely divided

into two elongate lobes, which Newstead (1911 *b*) has called the inferior claspers.

The true *ninth sternite* has been incorrectly spoken of by me (1914) as the ninth tergite, and equally incorrectly by Leicester (1908) as the sur-anal plate. It exhibits fewer modifications in the Culicidae than does the tergite, being usually represented by a narrow strip of chitin. In some *Anopheles* it is apparently absent; in *Culex perfdiosus* it is greatly enlarged; in a few species of *Aedes* (sub-genus *Aedes*) it has developed lateral processes, and in some forms it is strongly emarginate at the tip. It usually bears a few bristles about the middle, and in *Armigeres obturbans* is chitinated only at the sides and round these bristles.

The ninth sternite is well developed in most Limnobiidae, and in *Trichocera*, but in Psychodidae and some Mycetophilidae it is not distinguishable; in many other Mycetophilidae, in Bibionidae and in *Tipula*, it is very large and forms a single piece with the basal joint of the forceps (see below). In Cyclorrhapha it is said by Awati (1915) to be absent, but may possibly be represented by his vesiculum or by the editum of Newstead (1911 *a*). However, it is very difficult to homologise the part of the Nematocera with those of the Cyclorrhapha, and it is beyond the writer's intention and competence to do so.

(2) THE APPENDAGES OF THE NINTH SEGMENT. In all mosquitoes, as well as in the majority of the more primitive Diptera, the ninth segment bears a pair of two-jointed* appendages, which articulate with the ninth sternite, usually at its attenuated sides. Appendages of the ninth sternite occur in other orders of insects; they are universal in Lepidoptera, where the term *harpagones* was proposed for them by Buchanan White (1878); by P. H. Gosse (1883) they have been styled *valves*, and by some Lepidopterists *harpes*. The first and third of these terms have been used in varying and contradictory senses by writers on Culicidae, but never for the appendages of the ninth sternite; they are not in use by other Dipterists, and it is inadvisable that they should be retained. The term *valves* is quite inappropriate, at least in regard to the Diptera, and it also must be rejected.

* Three-jointed if the terminal spine is reckoned as a joint.

The term *forceps* (*forcipules* of the French writers, *Zange* of the Germans) has frequently been employed, and will be useful when it is desired to speak of the whole appendages.

(a) *The basal joint.* The homology of this piece is uncertain. By many it is regarded as simply a specialised part of the ninth sternite, and there is much to be said in justification of this view. As stated above, in many Nematocerous Diptera, including most Mycetophilidae and Bibionidae, the part cannot be distinguished from the ninth sternite, though it is quite possible to argue that this may be due to a secondary fusion. The apical part of the sternite in these cases has usually a median furrow or slit which may indicate either incipient division or incomplete fusion. Another point to be noted is that in some cases where the ninth sternite is apparently separate from the basal joints of the forceps, these latter are connected at their bases ventrally (e.g., *Trichocera*, *Macrocera*). In Limnobiidae the sternite and basal joints of the forceps are usually well separated.

In view of the uncertainty as to the exact homology, and pending further investigation and discussion, it will perhaps be as well to avoid using the term 'basal joint of forceps.' This is the more desirable since other terms are in use by students of Nematocera. Snodgrass (1904) proposed *pleuron*, while Dyar and other writers on the Culicidae speak of it as the *side-piece*. Both these terms are good, but Snodgrass's suggests the implication that it is derived from a separate pleural piece analagous with the thoracic pleura: perhaps a correct assumption, but as yet unproven. *Side-piece* is more non-committal, and is widely used by writers on Culicidae. There seems, therefore, to be no occasion for replacing it.

This part is subject to many important modifications in the different genera of the family. In most it has the form of a hollow chitinous tube, widely open at the base, especially on the inner side, and tapering more or less to the tip. Sometimes, however, the chitin is discontinuous on part or the whole of the inner aspect of the tube, where the wall may be formed of thin membrane only. *Anopheles* and *Chaoborus* have the tube complete, or almost so; some lacunae of chitination appear in some of the early Culicine genera, notably *Megarhinus*, the final stage being reached in the *Aedes* group, of which it is highly characteristic. Here the side-

piece forms a *lower flap** (ventral in actual position) and an *upper flap* (dorsal) connected on the outside by chitin and on the inside by thin membrane, which extends right up to the tip.

The apical and basal lobes of the side-piece, of which so much use has been made in the classification of the *Aëdes* group by Dyar (1918) are developments at the apex and base of the lower flap of the side-piece. The terms are appropriate, and I do not propose to alter them, but it may be worth while to point out that the basal lobes of *Megarhinus* seem to be developed from the upper flap of the side-piece, and further that the basal lobes in *Uranotaenia*, *Theobaldia*, etc., which have the chitin of the side-pieces tubular, may not be homologous with the basal lobes of *Aëdes*. The ventrally-directed, *sub-apical lobes* of *Culex* seem to be an independent development, as they can be traced back to *Theobaldia*, where they originate as simple hairy knobs quite independent of the basal lobes. The latter have disappeared in *Culex*, the sub-apical lobes having probably taken on their function, whatever that may be.

In the *Aëdes* group a characteristic modification of the base of the *upper flap* of the side-piece occurs, which in the great majority of species has become practically a distinct organ. For this part the term *harpe* was used by Felt (1905), though that author had very imperfectly studied the homologies, and used the same term for the anal chitinisations of *Culex*. Howard, Dyar and Knab (1912) adopted Felt's terms in restricted and differing senses, and spoke of the parts now in question as *harpagones*. Both these terms, however, are unfortunate and misleading, especially the latter, which, as has already been stated, was originally applied by Buchanan White to the main clasping organs of the Lepidoptera, which are probably equivalent to the side-pieces. The name *harpe* was first introduced by P. H. Gosse (1883) for an internal appendage of the side-piece (harpago), and there would, therefore, seem to be some justification for the use Felt made of it in the Culicidae. However, I consider that the harpe of the Lepidoptera (in the sense of Gosse) is more likely to be the equivalent of the second joint of the forceps of Diptera, than of the special organs in

* It will be well to avoid the terms *dorsal* and *ventral* as far as possible to save possible confusion.

consideration which are only found in the genus *Aedes*. Moreover, the term has been used so inconsistently in the Culicidae that its retention is undesirable without strong reason.

The term *claspette* was used in a loose way by Felt, apparently to cover any appendage, basal or apical, of the side-pieces, other than the clasper. Since the special organs of *Aedes* are undoubtedly homologous with the basal lobes of some forms (if not of all), there can be no objection on morphological grounds to the use of the term *claspette* for them, and no confusion will result from its re-introduction, as it has not been in general use. I propose, therefore, to make use of it for the harpagones (Dyar) of *Aedes*, and for the similarly derived structures of *Taeniorhynchus*, without necessarily intending to imply a strict homology between these two. The same name may also be applied to the parts in *Anopheles* which Christophers (1915) has spoken of as the harpagones, since it seems most probable that they are, as he suggests, homologous with the claspettes (harpagones) of *Aedes*. The *claspette spines* of Christophers would be better known as the *basal spines*, since they are not borne by the claspettes and may well be homologous with the basal lobes of *Aedes*. The term *basal lobes* I propose to reserve for the structures at the base of the lower flap of the side-piece in *Aedes*, and for the hardly differentiated organs of some other genera (*Megarhinus*, *Theobaldia*, etc.).

Brolemann (1919 b) speaks of the claspettes as *gonapophyses*, and regards them as appendages of the tenth tergite, but this is merely owing to his fundamental misconception as to the anus and the genital opening. He has further been led to the erroneous deduction that the claspettes are primitive organs which have disappeared in *Culex* and other genera, whereas they are unquestionably special developments (most probably arising quite independently) in *Aedes*, *Anopheles* and *Taeniorhynchus*. I prefer not to use the term *gonapophyses*, as it is a loose one used by different writers to indicate appendages of the ninth or tenth segment or of the genital tube.

Before leaving the side-pieces, it will be necessary to notice one other structure which has escaped the attention of most writers on the subject, Christophers excepted. This is an internal prolongation of the base of the side-piece on the lower (anal) side, variously

developed in different genera. I propose to term it simply the *apodeme*. Its importance will appear later on.

(b) *The second joint*. This I have spoken of as the *clasper*, and this term seems to be in fairly general use, though some writers, Christophers for example, have used it to include the basal joint or side-piece also. I do not think this fact need prevent its retention in the sense in which I have employed it. There has never been any doubt as to its homology throughout the Nematocera, unless perhaps in one or two aberrant species of *Aedes*, but its modifications are extreme. On this account I object to the term *clasp filament*, which has been widely used by Howard, Dyar and Knab: the structure is only 'filamentous' in certain groups, the description being absurdly inapplicable to such elaborate developments as are found in *Sabeihes*, and many *Aedes*.

Berlese (1906) calls this part the *mesostylus*; Snodgrass (1904) the apical appendage, and de Meijere (1919) the *stylus*. The first two of these are not sufficiently expressive; no objection need be made to the third, but *clasper* seems to me preferable as having been in more general use by the English writers. In those Diptera (such as the majority of Limnobiinae and Mycetophilidae) where it is double it will be useful to speak of the *upper* and *lower claspers*, though it is possible that one part may have originally been a terminal joint, or a mere lobe of the other. This does not apply to mosquitoes. The position of the plane of articulation of the clasper is important. It is vertical, or nearly so, in *Culex*, almost horizontal in *Aedes* (sub-genus *Ochlerotatus*).

The *spine* or *claw* of the clasper is best spoken of as such (*claw* being rather better than *spine*). It has been regarded by Brolemann (1919) as a third joint of the forceps, but this is not established. It may be *terminal* or *sub-terminal*, or rarely absent.

(3) THE ANAL CHITINISATIONS. By most writers the anus of insects is regarded as opening at the tip of the tenth abdominal segment, though Berlese (1906) recognises an additional segment near the base, and so counts the anal segment as the eleventh, while Keilin (in conversation) considers the anus to be situated on an appendage of the ninth, and does not admit a separate anal segment. At present, I believe that the majority in this case are right, and I therefore propose to follow the usual custom and speak

of the plate or plates dorsal to the anus as the *tenth tergite* or *tergites*, and those ventral to it as the *tenth sternites*.

In many insects the tenth tergite bears a pair of appendages (the anal styles, stylets or cerci), but this is not the case in the males of any Culicidae. Both tergite and sternite, however, are generally divided completely into two parts. The *tenth tergites* have not, so far as I am aware, been noticed by any previous writer* on the family, though they are quite well developed as two simple plates in most of the genera, particularly in *Theobaldia* and *Culex*. Apically they are in contact with the tenth sternites, basally with the ninth tergite. Owing to the torsion, they, of course, occupy the most ventral position in the hypopygium.

The *tenth sternites* are conspicuous in most Culicidae, and are the parts miscalled *harpes* by Howard, Dyar and Knab and 'bras peniens' by Brolemann. Their true nature is shown in any lightly macerated specimen by the fact that the rectum is attached to them (and to the tenth tergites); furthermore, they nearly always bear some minute bristles, which illustrates their cuticular origin. Though this is not true of all other insects, bristles seem never to occur on the genital chitinisations of Diptera.

A condition of the anal segment very similar to what is found in the Culicidae occurs in the Cyclorrhapha. In *Glossina* the halves of the segment have been spoken of by Newstead (1911 *a*) as *superior claspers*, but the true homology of these parts has been pointed out by Awati (1915), whose opinion I can confirm. In *Phlebotomus*, however, Newstead (1911 *b*) calls the anal segment the sub-median *lamellae*, and uses the term *superior claspers* for the forceps. This is no doubt owing to his having overlooked the torsion, which is as regular a feature of *Phlebotomus* as of the Culicidae, and is one of the points of agreement indicating a connection between the two groups. In Mycetophilidae, Bibionidae and Cecidomyiidae the anal segment usually has a different form, the tergite being divided into two hairy lamellae resembling the cerci of the female, while the sternite remains entire.

The most primitive conditions of the anal segment is probably that in which both the tergite and sternite are simple. This is the case in *Chaoborus*, though here the tenth tergite is fused on to the ninth. A very similar condition occurs in the Lepidoptera (see

* They are indicated in some of my published figures, and also by Brolemann, but have not been specially mentioned.

below) and also in Chironominae. In *Anopheles* there is often practically no chitinisation either of tergite or sternite, the whole segment being membranous; in some species two ill-defined bars of chitin represent the sternite. The same is true of *Aëdomyia*, and in these cases Christophers' term *anal lobe* is appropriate.

In other Culicidae the form of the anal segment varies little, except in *Culex*, where the sternites develop crowns of spines and in many cases a strong *basal arm*, projecting upwards and more or less surrounding the aedoeagus. In their primitive condition (as regards the Culicidae) the tenth sternites probably bore apically a few strong bristles or teeth (as in *Theobaldia* and *Taeniorhynchus*); the number of these has greatly increased in *Culex* to form the crown of spines, while in *Aedes* they have entirely disappeared. In addition to these spines there is nearly always a patch of a few very minute hairs, doubtless sensory in function, at the sides near the tip; these occur in *Aedes* as well as in *Culex*. In all cases where they are well developed, the sternites have a downward projection in the form of a strip of chitin extending up to and articulating with the lobes of the ninth tergite (figured by Felt, 1905, p. 465). Whether this is a primitive or secondary structure I am unable to say. It does not appear to exhibit any characters which can be made use of in classification, and it will, therefore, be unnecessary to name it.

Dyar (1918) speaks of the anal sternites (harpes) as evolving from a simpler to a more highly developed form, and regards their absence in *Anopheles* as primitive. It is quite certain, however, that the reverse is true, and that this absence is due to degeneration.

(4) THE GENITAL CHITINISATIONS. As in the case of most other insects, the genital tube of mosquitoes opens between the ninth and tenth sternites, and its apical part, with one or more invaginations, is more or less heavily chitinised. These chitinisations have been called collectively by Felt (1905) and Howard, Dyar and Knab (1912) the *unci*, though in the case of *Culex* these writers have also spoken of parts of them as *harpagones*.

The inapplicability of the latter term has already been explained. The name *uncus* was first introduced by P. H. Gosse (1883) for the 'posterior part of the dorsal arch of the eighth segment' of the Lepidoptera, and has been in use ever since for the same structure,

which, however, was incorrectly described by Gosse as belonging to the eighth segment. It has since been homologised by Rothschild and Jordan (1904) as the tenth tergite. The term *unci*, therefore, ought not to be used for structures belonging to the genital tube. Brolemann's term 'cylindre perianal' is, of course, out of the question, being founded on a misconception.

Christophers (1915) and Awati (1915) have adopted the term *theca*, which was first introduced by Wesché (1906) for the 'penis sheath' of certain Diptera, chiefly Cyclorrhapha. I reject this, however, as it is not by any means in general use among students of other groups, and the homology of the pars in Cyclorrhapha is still very uncertain. The alternative term *admniculum* of Westhoff (1882) has not often been adopted. The whole structure is often spoken of, perhaps correctly, as the *penis*, but I consider that this term would be better reserved for the actual intromittent organ when present. On the whole the term which seems open to the least objection for the chitinised parts of the genital tube is *aedoeagus*, which, according to Sharp and Muir (1912) was introduced by Foudras in 1859. It is in general use among Coleopterists in the sense indicated, but, unfortunately, has been employed by Pierce (1914) and other Lepidopterists in a much more restricted sense, to indicate the intromittent organ. I consider that Sharp's usage is the one that should be followed, and propose to adopt the name *aedoeagus* for the *ensemble* of chitinous structures of the genital tube of Diptera, thus exactly reversing Pierce's use of the terms penis and aedoeagus.

The apparent correspondence between primitive Diptera and primitive Coleoptera in the details of structure of the aedoeagus is remarkable, and comparable modifications have also been described for the Anoplura and Mallophaga. There is, of course, an immense divergence between the more specialised species, but in the more primitive forms, among which may be included the majority of the Culicidae, it is possible to distinguish the following parts:—

(a) *Basal plates*. These are a pair of chitinisations of the sides of the genital tube lying within the ninth segment and often extending back into the eighth. They doubtless serve for the attachment of muscles. Although generally overlooked, they have been mentioned by Dyar (1918) as *ligaments* and by Brolemann

(1919 *b*) as *apodemes aliformes*. The name *basalplatte* was proposed by Verhoeff (1893) for the corresponding structure in the Coleoptera, where, however, it usually forms but a single piece. I propose to adopt this name, using it in the plural, as the structures are apparently always paired in the Culicidae. Sharp and Muir (1912) prefer the expression *basal piece*. Waterston (1914) and Cummings (1916), on the other hand, use *basal plate*.

The basal plates vary much in size, attaining their maximum in *Culex* and *Theobaldia*. They articulate near their inner extremities with the apodeme of the side-piece, and it is noteworthy that the size of the apodemes varies inversely with that of the basal plates. In very many cases (conspicuously in *Theobaldia longiareolata*) there is a definite fusion between the basal plate and the apodeme, so that it is impossible to say where one ends and the other begins. In *Uranotaenia* this fusion is for almost their entire length. At their outer ends they articulate in a notch situated near the middle of the parameres, and they are also in close contact, though never fused, with the tenth sternites.

(b) *Parameres*. These are paired structures which, as just mentioned, articulate with the basal plates. They are nearly always present, though in varying degrees of development, being very minute in *Aedomyia*, and perhaps absent in *Anopheles*. They were formerly referred to by Dyar and Knab (1909) (in *Culex*) as the fourth plate of the harpagones; more recently by Dyar (1931¹⁸) as the first uncal plate. Brolemann (1919 *b*) calls them *trigonapophyses*. Berlese (1906), Snodgrass (1904) and de Meijere (1919) use this term *gonapophyses*.

The term *parameres*, which I have adopted as most suitable, was proposed by Verhoeff (1893) for the Coleoptera, and has been adopted by Waterston (1914) and Cummings (1916) for the Siphonaptera and Mallophaga respectively. While in these different orders the structures may not be strictly homologous, they are certainly similar in position and appearance. Sharp and Muir (1912) prefer the expression *lateral lobes*.

In some cases, though not in any mosquitoes, the parameres are fused with one another and with the basal plates. When this is so, Sharp's term *tegmen* may aptly be applied to the whole structure. This seems to be the condition in the genus *Molophilus*.

(c) *Mesosome*. Lying between the parameres is a more or less complicated body which has been incorrectly styled the *unci* by Howard, Dyar and Knab (1912), or the second, third and fourth uncal plates by Dyar (1915). Though apparently composed, especially in *Culex*, of one or more pairs of distinct organs, a close examination will nearly always show that it is really only one piece, more or less elaborately lobed. The very handy term *mesosome* was proposed by Waterston (1914) for the structures lying between and distal to the parameres of the Philopteridae (Mallophaga), and I propose to make use of it. I think it is preferable to the *median lobe* of Sharp and Muir (1912) since the structure often bears many lobes or divisions.

The mesosome is a thickening of the walls of the distal part of the aedoeagus. The chitin seems generally to be disconnected on the upper (apparent dorsal) side, but the lateral portions are generally, if not always, connected by a chitinous *bridge* (a good term used already by Dyar) on the side nearest the anus. This bridge varies much in width in different species, being extremely broad in *Aedes* subgenus *Ochlerotatus*, and very narrow in many species of *Culex*. When the halves of the mesosome are connected by chitin on the dorsal and ventral sides of the genital tube, the bridge nearest the rectum may be called the *lower bridge*, and that furthest from it the *upper bridge*. The terms dorsal and ventral would be ambiguous, and had best not be used. The two bridges are both distinct, though narrow, in *Culex*. In the higher members of this genus there are two or more fairly distinct parts of the mesosome, which seem to be developed mainly or entirely on the upper wall of the tube, and probably do not indicate an invagination of the tube. When a second invagination is present* the terms *endomere* and *telomere* have been used by Waterston (1914) and Cummings (1916) for the proximal and distal portions of the mesosome. I have not been able to satisfy myself, however, that any such division occurs in the Culicidae, even among the more complicated forms.

The structure of the mesosome is of great taxonomic importance, and there is probably no other single organ of the body which gives better clues to the true phylo-genetic relationships of the species.

* The first is at the base of the parameres.

It is beyond the intention of this paper to discuss its modifications in detail, but it may be mentioned that in *Culex* the bridge or bridges are nearly or quite basal, the part beyond them being drawn out into horns or spines, usually on the upper side of the tube. In *Aedes*, on the other hand, as in many other genera, the lower bridge is almost or quite apical; as it is also much broader, the mesosome of these forms is necessarily practically a rigid structure, and is only capable of slight extension by the action of the parameres, conjunction with the female being effected mainly by the aid of the mobile forceps. In *Megarhinus* the lower bridge occupies a middle position, and in this as in some other points this genus should probably be considered among the most primitive of the family.

The true genital structures of *Anopheles* are very different from those of other Culicidae, and are difficult to homologise. It is almost impossible to distinguish parameres, basal plates and mesosome as can be done in the other genera of the family. The first two appear to be represented by a small single piece no larger than the paramere of *Aedomyia* (see fig. 2, c). The mesosome is Christophers' *theca*, but it is of a very different form from that of any of the other genera. A very careful study of cleared and floating specimens of some of the more generalised *Anopheles*, such as *A. plumbeus* or *A. argyrotarsis*, shows that it approaches nearest to the simple form seen in *Ochlerotatus*, with a very broad lower bridge. In most species, however, the chitin has become definitely tubular and the remarkable and characteristic *leaflets* have been developed at the tip.

The structure of *Chaoborus* is also very different from that of the Culicinae, and here there would seem to be no definite aedoeagus. There is only one pair of chitinous organs at the base of the side-pieces, occupying a vertical position and possibly to be regarded as developments of the side-pieces themselves. A similar condition occurs in the Chironomidae (excepting Ceratopogoninae), and in both cases is most probably retrogressive. It is possible that a connection may be indicated between the Culicidae and Chironomidae through *Chaoborus*, which, indeed, would also seem likely from a study of some other organs.

Hardly anything is yet known as to the precise function of the different parts of the hypopygium, but one point which is clear from

a study of macerated specimens of *Culex* may be mentioned, as it is of some general interest. The parts of the aedoeagus (see Edwards (1914)) are observed in microscopic preparations in two different positions relative to one another. In one, the position of rest, the halves of the mesosome lie close together, project tailwards, and are partly covered by the parameres. In the other, probably the position of use, the parameres are folded back and the halves of the mesosome either divaricated from the bridge outwards or bent downwards on to the anal sternites. A comparison of specimens in these two positions (the examination should be made in clove oil under a binocular microscope) will show that in the first the upper bridge of the mesosome lies proximally to the lower, and both are almost in the line of the longitudinal axis of the body, the aperture of the genital tube thus being closed. The folding back of the parameres has the effect of pulling back the upper bridge of the mesosome, so that it and the lower bridge lie in a plane vertical to the body, and at the same time divaricating the halves of the mesosome (which lie distal to the bridges and are not connected by membrane), and turning them downwards. The result is not only the genital tube is opened, but that the lower bridge, or the whole mesosome presses on the tenth sternites and thus closes the anus, probably at the same time transmitting some stimulus by touching the minute sense-hairs on the tenth sternites. A quite analogous condition has been described by Waterston (1909) in the Siphonaptera, but I have not seen anything approaching it in other genera of mosquitoes.

The accompanying diagram (fig. 1) will help to explain the

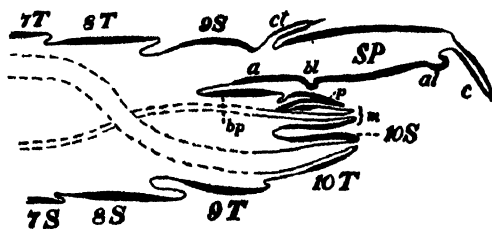


FIG. 1

Ideal section through tip of abdomen of an *Aedes*. The thick lines represent chitinisations, the thin ones membrane. Note the wide membrane between the 8th and 9th segments, which allows of the torsion. 7T—10T, seventh to tenth tergites; 7S—10S, seventh to tenth sternites; SP, side piece; c, clasper; ct, claspette; bl, basal lobe; al, apical lobe; a, apodeme; bp, basal plate; p, paramere; m, mesosome.

foregoing analysis of the Culicid hypopygium. It represents an ideal longitudinal section through the tip of the abdomen of an *Aedes*, this type being chosen as offering the maximum development of all the structures. All the parts shown are, of course, not actually in the same vertical plane, and the claspettes and parameres are shown dorsal to their true position for the sake of clearness. The course of the rectum and ejaculatory duct is indicated by dotted lines, since it is not quite certain what happens to the internal organs after the torsion. If this diagram be compared with one given by Cummings (1916, p. 688) for the Mallophaga, it will be evident at once that there is a striking similarity in many respects. Camera lucida drawings are also given of the aedoeagus of *Megarhinus*, *Ochlerotatus*, *Aedomyia* and *Theobaldia* (fig 2, A-D), in order to show the principal types of mesosome.

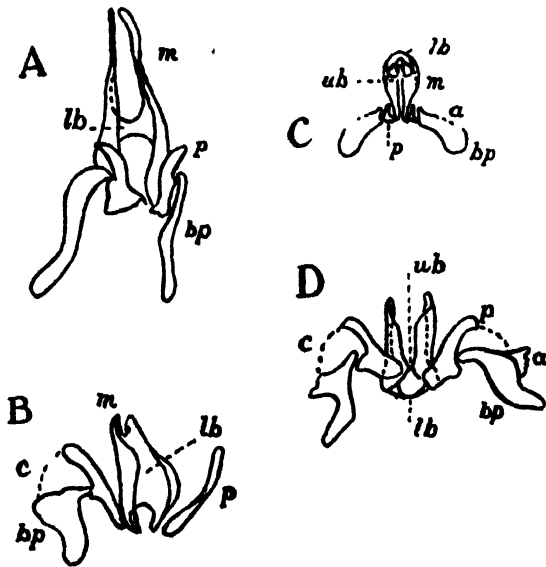




FIG. 2

Aedoeagus of different Culicidae: A, *Megarhinus* (*Toxorhynchites*) *regius* (Tennent); B, *Aedes* (*Ochlerotatus*) *waterhousei* (Theo.); C, *Aedomyia* *squamipennis* (Arrib.); D, *Theobaldia* *annulata* (Schränk). a. position of attachment or fusion of apodeme with basal plate; bp, basal plate; p, paramere; m, mesosome ub, upper bridge, and lb, lower bridge of mesosome; c, membrane connecting the outer edge of the paramere with the basal plate.

To sum up, a table is also given shewing the chief terms which have been used by different writers on the Culicidae, and for comparison, the equivalent terms used by Newstead (1911 b) for

Phlebotomus and by de Meijere (1919) for the *Tipulidae*. The last-named author compares his nomenclature with that of Berlese, Snodgrass and Westhoff, so that it will be unnecessary to add their terms to this table; reference may be made to de Meijere's work.

In conclusion, I wish to express my indebtedness to Dr. D. Sharp and Capt. J. Waterston for much kind assistance on difficult points.

| Proposed Terms | Felt, 1905 | Dyar, 1918 | Brolemann, 1919 | Newstead, 1911 | de Meijere, 1919 |
|---------------------|---|---|----------------------------------|------------------------------------|---|
| Ninth tergite ... | Setaceous lobes | Basal appendages | Ninth sternite | Inferior claspers | Ninth tergite |
| Ninth sternite ... | — | — | Ninth tergite | — | Ninth sternite (proximal part) |
| Side piece ... | Basal joint of clasp | Side piece | First joint of forceps | Superior clasper (basal joint) | Basal joint of forceps (part of ninth sternite) |
| Basal lobes ... | Claspette | Basal lobes | Verrue basale | — | — |
| Apical lobes ... | Claspette | Apical lobes | Saillie apicale | — | — |
| Claspettes ... | Harpes | Harpagones | Gonapophyses of 10th sternite | Intermediate appendages | — |
| Apodeme ... | — | — | — | — | — |
| Clasper ... | Second joint of clasp | Clasp filament | Second joint of forceps | Superior clasper (second joint) | Stylus (terminal joint of forceps) |
| Tenth tergites ... | — | — | — | } Submedian lamellae | Tenth tergite |
| Tenth sternites ... | } Harpes (<i>Culex</i>) Harpagones (<i>Aedes</i>) | Harpes | Bras penien | | Tenth sternite |
| Aedoeagus ... | Unci ? | Unci | — | — | — |
| Basal plates ... |  | Ligament | Apodème aliforme | } Intromittent organ | — |
| Parameres ... |  | First plate of unci (<i>Culex</i>) | Trigonopophyses | | Gonapophyses |
| Mesosome ... | Harpagones (<i>Culex</i>) | Second to fourth plates of unci (<i>Culex</i>) Unci | Cylindre perianale | Genital filament | Penis |

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STUDIES ON THE VARIOUS TYPES OF MALARIAL INFECTION AND THE EFFECT OF QUININE TREATMENT THEREON AMONG THE NATIVE POPULATION OF THE MALAY ARCHIPELAGO

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I. INTRODUCTION

During the course of our researches on malaria in the malarial districts of the Malay Archipelago, we have examined the blood of numerous natives, including those who were being subjected to quinine treatment. We were induced to do so on the following grounds:—Our primary object was to ascertain the percentage of different species of Anophelines showing malarial infection in nature, and we soon found that no comparison could be made between the results obtained in different localities without having some definite notion regarding the number of gamete carriers in each district.

The result of blood examination in various localities revealed, however, some facts which seemed worthy of record, particularly in their bearing on quinisation and its effects.

In the following pages we will give a short account firstly of malaria in the Malay Archipelago, secondly of the types of malarial infection found by blood and spleen examinations, and thirdly of the changes observed in these types by quinine treatment.

II. MALARIA IN THE MALAY ARCHIPELAGO

There are numerous places where malaria is endemic, *e.g.*, in many parts of the north coast of Java, where the spleen rate is continuously high, but the morbidity (as expressed by the admissions to the hospitals) shows considerable fluctuations. In Semarang, in 1917, the rate of admission was very high, and in 1918 it sank almost to zero. Still this does not indicate that the malarial infection was a low one, examination of the native population disclosing a spleen rate of 80 to 90 per cent., and a parasite rate of 24 to 38 per cent. in children. This shows how little value can be attached to the admission rate as a measurement of malaria. Still this rate is an indicator of some sort, and when in one year there are more admissions than in another, we may assume that the frequency of malaria has been altered likewise.

Not only is there a fluctuation in the amount of malaria from one year to another, but, as in other countries, this fluctuation is seasonal, and it is a striking feature that most cases occur (and usually the mortality is at its height) in July-August, during the dry season (Chart 1).*

This rule generally holds, but there are exceptions. As an instance, we found a severe epidemic in Kepetakan (north coast of Java) in May and June (beginning of the dry season), and one in Soendatar (Sumatra) in April and May (end of the wet season).

Although there may be many transitional forms, we may distinguish: (1) *Endemic malaria*, maintaining a high spleen-rate all the year round, showing each year an exacerbation in the periods mentioned above, and especially severe exacerbations in periods separated by an interval of a varying number of years. (2) *Epidemic malaria*, *i.e.*, periodical severe outbreaks in districts where, as a rule, this disease is rare.

* This is a well-known fact in our colonies. We emphasize it, because it is usual to cite Koch's report (1900), when referring to malaria in the Dutch East Indies. This report states that in these regions malaria is most frequent at the beginning and the end of the dry season (October to November, April to May). This erroneous statement led Koch to the conclusion that malaria was not frequent in Batavia, because in the off-season (October to November), which was supposed by him to be the malarial season (beginning of the wet monsoon), he found only a comparatively small number of cases. This conclusion in its turn made him praise the Government for their gratuitous quinine distribution among natives, which, according to Koch, had caused the marked reduction of malaria.

The general tendency of the outbreaks to occur in the dry season, is a feature especially noticeable along the north coast of Java, but also to be observed in the interior. Probably this tendency has various causes, but one at least we have been able to elucidate, viz., the periodicity of the Anophelines.

Along the coast in Java and Sumatra, *M. ludlowi* has been shown by us (1918, 1919) to be the principal carrier. It breeds, together with *M. rossi*, in the salt-water ponds along the coast, overgrown with algae (*Enteromorpha*, *Cladophora*) and other filamentous weeds (e.g., *Najas* sp.). These pools contain a mixture of fresh and salt water and, as the dry season proceeds, they become more and more brackish, as has been shown by v. Breemen (1919). In June to August, these pools are excellent breeding-places for *M. ludlowi* and *M. rossi*, which occur there in enormous numbers, hundreds of larvae being gathered at one dip. But at the end of the dry season (September, October, sometimes as early as August) the larvae disappear completely, as we have noted in Tegal, or only *M. rossi* remains, as was the case in Semarang. V. Breemen has shown that this occurs when the salinity of the water exceeds a certain amount of NaCl. The malarial frequency is much reduced, shortly after *M. ludlowi* becomes scanty or disappears.

In the interior *M. ludlowi* is usually absent (except in some parts of Sumatra, e.g., Mandailing, where this mosquito exists all the year round, although in somewhat varying numbers) and the principal carrier there is *M. aconita* (sometimes *N. maculatus* or *N. leucosphra*). Except in very small islands, there is always plenty of water at the disposal of the larvae, and so it is not clear why *M. aconita* (in a station which we observed for almost a whole year) should not breed permanently, as its larvae were found in the rice-fields, which are inundated from January till July and from August till November. The fact is, that it was only observed in great numbers (as larvae and adults) during the height of the dry season (June, July) at the time of the cutting of the first crop of paddy; the conditions prevailing in mature rice-fields, ready to be harvested were, in this instance, particularly favourable for the breeding of this species.

III. THE TYPES OF MALARIAL INFECTION AMONG THE POPULATION IN THE MALARIAL DISTRICTS

Before describing these types, it will be useful to explain some terms used here. We distinguish between the 'infection-rate' and the 'parasite-rate.'

The infection-rate is :—

$$\frac{\text{number of tertian + quartan + subtertian-infections}}{\text{number of persons examined}} \times 100$$

The parasite-rate is :—

$$\frac{\text{number of parasite-carriers}}{\text{number of persons examined}} \times 100$$

The first rate may be greater than the second if mixed infections are present (Table XVIII). It may also surpass 100 per cent.

1. The endemic type

This type occurs in localities of high and continuous endemicity. The spleen rate is high in children and remains so in adults (Table I

and Chart 2). This fact,* first recorded by Schüffner (1919) is remarkable, as it is generally asserted that the spleen rate should be taken in children under ten years of age; the splenic enlargement rapidly decreasing in adults.

The infection rate, on the other hand, shows the well-known reduction in older children and in adults. It reaches its maximum in the 4-5 year period, and always remains much below the spleen rate. The decrease of the three species of parasites does not proceed with the same rapidity, simple tertian and quartan usually disappearing sooner than subtertian, and the crescents sooner than the subtertian asexual forms (trophozoites). This latter fact causes the ratio of the number of crescent carriers to the total number of subtertian cases (referred to in the following pages as $\frac{c}{t}$) to diminish (Table I and Chart 2).

Darling (1911) showed that 1 crescent per 500 leucocytes in the blood is the smallest number capable of infecting Anophelines. We use the term 'highly infective' carriers for those showing 1 or more crescents per 100 leucocytes. These highly infective carriers are more numerous among children than among adults in endemic regions (Tables I, V and XIV and Charts 2 and 4).

Mixed infections occur both in adults and children, but in the latter they are more numerous. In adults they may even be absent (Table XVIII).

There is a certain amount of confusion regarding the part played by immunity in the production of gametes. On the one hand, all observers agree that in endemic regions the children are heavily infected, the adults very slightly so or not at all. Here immunity causes the disappearance of all parasites and consequently, of the gametes also. On the other hand Koch (1900) states that incipient immunity causes a condition, named by him 'latent malaria,' in which morbid symptoms are absent, but parasites, and especially sexual forms, are still present. This condition is found in elder children and adult immigrants, who have already suffered from numerous relapses and reinfections. Sergeant (1910) also mentions a similar state found in Algerian natives under similar conditions.

Thomson (1911) asserts that crescents are produced after the development of partial immunity. He bases this statement on the following facts (among others):

- (1) The ratio $\frac{\text{crescents}}{\text{trophozoites}}$ rises when the asexual forms diminish in number.

* We observed it everywhere in Java and Sumatra and also in many isolated localities, in the eastern portion of the Archipelago (even in New Guinea, Table XVII), in every way corresponding to the villages of Bongu and Bogajim, studied by Koch (1900), where the spleen rate at the age of 14 and over sank to zero.

(2) The number of crescents rises with the duration of the malarial infection, with the degree of splenic enlargement and with the age of the patient (if he has been constantly or frequently exposed to malarial infection).

It would thus seem that incipient immunity stimulates the production of gametes and that, consequently, they are more numerous in elder children. Our observations tend to show that in the Malay Archipelago the presence of crescents indicates the absence of immunity, as they are most numerous in younger children, decreasing in number with age, even more quickly than the trophozoites. The fact that the number of crescent-carriers arranged according to the degree of splenic enlargement rises and falls simultaneously with the rise and fall of the number of carriers of trophozoites (Table II) corroborates this view.

On the other hand we noted an inverse relation, between the numbers of trophozoites and crescents, in persons suffering from subtertian malaria (Table III). This fact, of course, would seem to support the view, that the formation of crescents is a reaction against the immune-bodies. The fact of the steady rise of the number of crescent carriers and of the value of $\frac{C}{t}$ from the beginning of the epidemic of Modjodjeder through four successive months (Table XIII, Chart 12) gives this an additional support.

We therefore conclude that if the production of crescents is at all stimulated by immunity, then it should be regarded as a very early symptom of the formation of antibodies, becoming manifest at a time when no other symptoms (fall of infection and spleen rate) are perceptible, and disappearing with the appearance of these latter symptoms. The cases of latent malaria (no morbid symptoms, with parasites still present) are either not crescent carriers at all or the crescents are present in negligible numbers only.

Adult emigrants from healthy regions, coming into endemic districts, show numerous crescents in their blood, just like children. These newcomers certainly form an additional danger to the community. We observed this by examining mosquitoes (*M. ludlowi*) in the house of one of them. Out of ten *M. ludlowi* caught there, seven were found to be infected; out of two hundred and twenty-nine *M. ludlowi* caught in the surrounding (native) houses of the same village, nineteen only showed infection. In another similar case, four *M. ludlowi* out of one hundred and one were found infected in the native village, and four out of six in the house of a European family, where two of five children harboured crescents in the blood (one crescent to ten leucocytes).

Exceptions to the rule

In Tandjong Pinang (Riouw Archipelago), a notorious malarious small town, as has been proved by the examination made by various medical officers, we found aberrant conditions. Judging by the distribution of subtertian, we had here a purely endemic type, as was

to be expected. But mixed infections (Table XVIII), tertian and quartan, were so numerous among adults as to resemble the epidemic type. The medical officer, Dr. Poser, who was in the habit of making blood films of every case of fever coming under his notice, informed us that in 1917 (the year preceding our survey) he found on an average sixty-five subtertian cases to thirty tertian and five quartan cases. We found this ratio to be 63 : 40 : 40. We conclude that the conditions for the spread of tertian and quartan had become much more favourable of late, among a population heavily infected with subtertian but not so with the other species, and consequently not immune against them. Of course, this explanation can only be accepted if we admit Koch's statement that immunity against one species of malaria parasite does not confer immunity against the others (Chart 3, Table IV). In Penjengat, another island of this Archipelago, we found similar conditions. Here another point is to be noted, viz., the low spleen rate of the adults. This is easily explained by the fact that the men mostly lived by fishing and went out to sea each night from 5.30 p.m. till 8.30 a.m. of the next day, many of them being in this way protected from infection, but at the same time prevented from maintaining their immunity (Chart 3, Table IV).

The most notable feature of the endemic type is the reduction of the crescent rate and of $\frac{c}{t}$ in adults. How is the latter to be explained? It may be due to the fact of immunity diminishing the power of the subtertian parasites to produce gametes, without killing the trophozoites. But it may be, also, that the number of trophozoite carriers is kept up to a certain level by continuous reinfection, but that the rings are being constantly killed, not so quickly as to remain imperceptible to microscopic examination but too quickly to produce gametes.

2. *The sub-endemic type*

Regions with high and continuous endemicity are often surrounded by others of less, but still continuous, endemicity, provided that the breeding-places of the transmitting *Anopheles* are not spread evenly over the whole country, but are localised. Such conditions exist everywhere on Java's north coast. Villages or

those quarters of towns which adjoin the salt-water marshes or salt-water fishponds, where *M. ludlowi* breeds, are highly malarious, but villages or quarters situated behind this zone are less so. Occasionally, however, there are severe outbreaks even there (partly at least caused by *M. ludlowi* penetrating farther inland than is its wont, as has been shown to be the case in Tegal and Semarang). When such outbreaks occur, a condition is realised somewhat intermediate between the epidemic and endemic type (Chart 4, Table V). The infection rate in adults is still much below that in children, but the tertian and crescent rates are comparatively high:— $\frac{c}{t}$ is equal in adults and children, so is the rate of infective crescent carriers. Usually the spleen rate is 30 to 50 per cent. in children and adults, 20 per cent. towards the periphery of the region. The infection rate is reduced correspondingly, but it is a curious fact, that the chart of blood examination arranged according to age (Charts 5 and 6, Tables VI and VII) shows the same features as in regions of high endemicity, *i.e.*, highest rate of infection in young children and very low one in adults. The decrease of the crescents in adults is likewise noticeable, but $\frac{c}{t}$ and the rate of infective carriers is not lower than in children. In Chart 5 we note a sudden rise in the infection, crescent and spleen rate at the age of 16 to 20. This is to be explained by the fact of the immigration of young coolies from non- or slightly malarious districts.

In comparing Chart 6 with Charts 2 and 5, we note another feature worth recording. In the regions of highest endemicity of Semarang (I, II, III) the infection rate reaches its maximum at the age of 4 to 5. In districts of lowest endemicity (IV) this maximum is reached at the age of 6 to 7. The lower rate of infection among the youngest children might be explained by the supposition that they are less exposed to infection than the older ones (personally, we believe this not to be correct). But this reason does not hold for children aged 4 to 5. Here another explanation is to be looked for, which may be found in the smaller chance of becoming infected. In the districts I to III, this chance is so great that the majority of children who become at all infected have contracted the disease one or more times before the expiration of the fifth year. But in district IV, this chance is much reduced, in consequence of which

it takes a greater number of years to reach the maximum infection rate.

3. *The epidemic type*

This is the type we met when an outbreak of malaria occurred in a place where the disease was said to be previously absent. Only once (at Modjodjedjer) we were able by personal examination to verify this statement.

The spleen rate of children and adults does not differ materially. It may be as high as or lower than that in endemic regions. In comparison with the spleen rate, the infection rate is much higher than in the latter areas, being equal to or surpassing the spleen rate. In consequence of this fact, the percentage of parasite carriers with non-enlarged spleens is much increased, especially at the beginning of an epidemic; later on the equilibrium is re-established (if no quinine is administered, *v. infra*) (Chart 14). There is no marked and constant difference in the composition of the parasite-fauna in the blood of persons of different age; the crescents are nearly as numerous in adults as in children, the rate of infective carriers differs slightly only, likewise the value of $\frac{c}{t}$. Contrary to the conditions prevailing in endemic regions, not only the children but the adults also are gamete reservoirs, in consequence of which the total number of infective carriers is much greater. This means for the Anophelines a greater chance of becoming infected. As an instance we mention that in endemic regions we found 3 to 4 per cent. of *M. ludlowi* infected in nature, whereas in epidemic regions the rate of infection was 16 to 35 per cent. An insignificant carrier like *M. rossi* showed a rate of infection of 0.3 to 0.4 per cent. in endemic regions, and one of 3 per cent. in an epidemic district (Charts 7, 8 and 9, Tables VIII, IX and X).

IV. CHANGES OBSERVED IN THE TYPE OF MALARIAL INFECTION UNDER THE INFLUENCE OF QUININE TREATMENT

It is generally supposed that quinine treatment among a population retards immunisation. This, at least, is Koch's opinion, who endeavours to explain away the difficulties opposed to his theory of immunity, by the effects of quininisation (1900). Another of these

effects is stated by Ross (1910) to consist in the reduction of the splenic enlargement without an accompanying disappearance of the parasites. As a third effect, James (1910) states that he has observed a rise in the number of crescent carriers.

As the results of a malarial survey are largely based on the blood and spleen examination, and as the facts mentioned seem to indicate that quinine treatment may modify to a considerable extent the value of the infection and spleen rate, we have endeavoured to elucidate the changes which occur in both indices in the course of a wholesale quininisation among a population, such as is usually performed only in times of severe epidemics. Our object in doing so was merely to prevent errors which might result from our ignorance as to the effect of this treatment.

We have observed this effect in three epidemics, where we had determined the infection and spleen rate previous to the commencement of the treatment. We will here deal with them separately.

1. *Epidemic in North Soendatar*

Data referring to the conditions before and after the treatment are given in Charts 10 and 14, and Tables XI, XV and XVI.

The campaign lasted for one month only. Quinine was given in cases of fever only, in the following doses, for one week at a time :—

Total population : 1264

| 0-2 years | 0.2 grammes | quinine-sulphate, p.d. | 9 cases. |
|-----------|-------------|------------------------|----------|
| 3-5 " | 0.4 " | " " | " 194 " |
| 6-12 " | 0.6 " | " " | " 185 " |
| 13-18 " | 0.8 " | " " | " 106 " |
| 19- " | 1.0 " | " " | " 379 " |

Individual treatment was continued for one week at least; for more if fever reappeared. Prolonged treatment may be enforced, but renders the whole proceeding highly unpopular, for which reason it is not encouraged by the authorities, and we had to do without it.

The changes in the spleen and infection rate after a month are insignificant. The spleen rate remains almost the same in children, but it is distinctly reduced in adults. The infection rate is reduced in both, but especially in adults. This is due to the

fall of the quartan infections, less so to the reduction of the tertian and subtertian infections. The crescent rate has been diminished slightly in children, more so in adults. The value of $\frac{c}{t}$ which, before treatment, was higher in adults than in children, has been reduced in the former. The rate of highly infective carriers has been decreased only slightly in adults and children (Table XVI).

The epidemic existed already for some months before the time of our survey and had attained a stationary condition. An equilibrium had been established between the spleen and parasite rates, *i.e.*, there were only a few parasite carriers without splenic enlargement. After the treatment, the percentage of parasite carriers not showing any palpable splenic enlargement had risen; more so in adults than in children (Chart 14, Table XI).

The fact that all the changes observed after the treatment are more marked in adults than in children may be ascribed to the fact that the first took more quinine.* It remains to be noted that, although there is no marked change in the splenic enlargement, the average enlargement has been much reduced (Table XV). Mixed infections, numerous in adults and children before the treatment, have decreased in number, especially in adults (Table XVIII).

In this and the following examples we compare the results of examination before and after treatment, without selecting the individuals known to have taken quinine. This was done in the cases of one hundred and five children and ninety adults (Chart 10a and Table XIa), but we did not observe any marked difference. From this we infer that the majority of fever patients really took quinine. This is satisfactory, but it should be remembered that in this and the other examples (except Margaredjo, *v. infra*) we have to do with epidemic malaria. The population is not used to it, is thoroughly frightened and glad for any help offered them. Blood and spleen examinations are no longer a favour they grant, but a succour which they gladly accept, believing it to be a peculiar form of preliminary treatment, which renders quinine more effective.

* But it may also be due to the nature of malarial infection in children rendering its treatment more difficult. The fact that it was so little effective in 105 children whom we knew to have taken quinine, seems to support this supposition. The experience at Margaredjo (*v. infra*) on the other hand, shows that children are not resistant to treatment.

2. *Epidemic of South Soendatar*

Conditions before and after treatment are noted in Charts 11 and 14, and Tables XII, XV and XVI. Method and period of quinisation are the same as in the preceding paragraph.

Circumstances were somewhat different from those existing in North Soendatar, because the epidemic was still on the increase during the treatment. In consequence of this, conditions were worse after the treatment than before. In children and adults the spleen and infection rates have risen, but tertian and quartan infections have been much reduced in adults, the increase of the infection rate as a whole being due to the subtertian infections. The crescent rate has risen in children and fallen in adults, the value of $\frac{c}{t}$ has been reduced in both, but especially in adults. This also holds for the rate of infective crescent cases. Mixed infections have decreased in both, but more markedly in adults (Table XVIII). The percentage of parasite carriers without enlarged spleen was already high before the treatment, and equal in adults and children. After treatment it has increased in both, but more in adults than in children. The reduction of the average splenic enlargement is well marked (although less so than in North Soendatar). This is remarkable, as the spleen rate has increased.

3. *Epidemic in Modjodjedjer*

(Charts 12 and 14; Tables XIII, XV and XVI.)

Quinisation was effected here (*a*) by house to house distribution, (*b*) by distribution at the missionary hospital clinic. The first was continued for about four weeks, the second for the whole time of the epidemic (June to September). As the natives there are used to European medication, even this method ensures a considerable use of quinine, but more among adults than among children. The dose for adults was 1·2 grammes of quinine sulphate p.d., for children 0·2 to 1·0 grammes. Two hundred and ninety of a population of six hundred and forty were treated.

Results were somewhat complicated by an outbreak of quartan malaria that appeared towards the end but which was absent at the beginning of the treatment, a fact offering a certain analogy to the

observations made in the Riouw Archipelago (cf. pages 57, 66). Moreover, the examination before the treatment was performed at a time when the epidemic was still increasing in severity, whereas the last examination occurred after the epidemic had reached its maximum.

The spleen rate in children and adults, almost equal at the onset, have both been altered, the first being increased and the second reduced. The crescent rate before the treatment was highest in adults. Afterwards it rose in children, in adults it almost sank to zero. Similar changes occurred in the tertian rate, whereas quartan became more numerous in adults than in children. The subtertian rate sank in both, but especially in the former. The value of $\frac{c}{t}$, which at first was equal in adults and children, afterwards rose in the latter and decreased in the former. The rate of highly infective crescent carriers remained stationary in children, but was much reduced in adults. During the time of our first examination, there still existed in adults and children numerous parasite carriers not showing splenic enlargement, the epidemic being at its commencement. After the epidemic had existed for about three months, the equilibrium was almost reached in children, only a small percentage of parasite carriers not suffering from splenic enlargement. But in adults this percentage was even higher than at the beginning. The average enlargement of the spleen has increased, even in adults, although the spleen rate has been reduced. We believe this to be explained by the fact that the average splenic enlargement is highest in quartan infections, and these occurred at the end of the epidemic (Table II).

Endemic malaria in Margaredjo

(Charts 13 and 14 ; Tables XIV and XVI.)

Judging by the former experiments, it might be inferred that a more or less considerable amelioration may be attained in adults, but that in children success is hardly perceptible. This is true if the treatment lasts for a comparatively short period only, but the quininisation of school children in Margaredjo shows that even in children results may be obtained.

The regular treatment of every child in the missionary school

has been continued by Dr. Bervoets for more than ten years. The successive generations of children have been trained so effectively that they come for treatment at each illness. When this proves to be malaria they undergo a complete cure for three weeks, and an after-cure for the time necessary to free them from excessive splenic enlargement and anaemia. The latter cure includes not only quinine but also iron preparations.

In conditions relating to malaria, Margaredjo resembles Samarang I-III. It is situated near the *ludlowi*-breeding salt-water marshes and fishponds. The spleen rate of the young children equals that of Samarang I-III. But at the time of our visit the infection and crescent rates of these children were much higher.

We divide the school children into three classes:—(1) Those which have been in the school and under treatment for one and a half to two years (aged 6-9); (2) for three to four years (aged 10-11); (3) for five to seven years (aged 12-13).

Under this régime infection and spleen rates diminish; at first tertian, quartan and crescents only, later on subtertian, which is the only, much reduced, infection still existing in the oldest class.

These changes, well marked by themselves, appear more striking when comparing the results of the examination of children in Margaredjo and Samarang (Chart 13). In both the infection rate gradually diminishes, but its composition is quite different. In Samarang there remains tertian infection (not to mention quartan, which in Margaredjo was scarce from the onset) and numerous crescent carriers, giving a high value to $\frac{c}{t}$. In Margaredjo we note a rapid disappearance of tertian and crescents, the latter not accompanied by a corresponding fall in the subtertian rate, rendering the value of $\frac{c}{t}$ a low one. The spleen rate, which in Samarang and Margaredjo was nearly equal in the younger children, remains constant or even rises a little in the former place, whereas it decreases considerably in Margaredjo. The percentage of parasite carriers without enlarged spleen was low in the younger classes of Margaredjo, as was to be expected in an endemic region, but it rose in the elder children. The reduction of the average splenic enlargement, although present, is not considerable. The only crescent carrier among the children of 10 to 11 years is non-infective (one crescent per three thousand leucocytes).

V. SUMMARY

If we try to formulate as concisely as possible the results of quinisation mentioned above, we may say that in epidemic districts it alters the nature of the parasite infection among the inhabitants, causing it to approach the endemic type, and this the more markedly and completely the longer the treatment (taken as a whole, not each case individually) lasts. This seems a poor result, but it is not so if the transformation is complete. It considerably reduces the chances of infection in such districts. This will become apparent when comparing the rate of natural infection of *M. ludlowi* in epidemic and endemic regions. In the latter we found 143 infected out of 5,613, *i.e.*, 2.6 per cent.; in the former 122 out of 611, *i.e.*, 20 per cent. The latter rate is so high because not only the children but the adults also include numerous infective gamete carriers, whereas in endemic regions only the children do so. The quinine treatment greatly reduces the number of crescent carriers among the adults, and we may expect that the chances for Anophelines to become infected are in that way reduced by a ratio corresponding to the above figures.

In endemic areas conditions are different. Here the children only are the gamete reservoirs, and if it is difficult to cure them in epidemic regions, it is doubly so in endemic. We would encounter fewer difficulties with the adults, but there is no use in treating them, because of their limited epidemiological significance. Here endeavours will be successful only under special and favourable conditions as in Margaredjo. Terburgh (1919) has vividly described the hopelessness of the prospect if such conditions are absent.

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TABLE I.

Blood and spleen examinations. Semarang I-II. Population: 13,687
(cf. Chart No. 2).

| Age in years | Number examined* | Spleen rate | INFECTION RATE | | | | | $\frac{c}{t}$ |
|-------------------|---------------------|----------------|-------------------|---------|-----------------|-----------|-----------|---------------|
| | | | Simple Tertian | Quartan | Sub- tertian | Crescents | | |
| | | | | | | Total | Infective | |
| 0- 3 | 39 (151) | 71 | 16 | ... | 20 | 15 | ... | ... |
| 4- 5 | 39 (107) | 81 | 13 | 7 | 31 | 18 | ... | ... |
| 6- 7 | 100 (228) | 79 | 8 | 7 | 12 | 6 | ... | ... |
| 8-11 | 111 (288) | 82 | 6 | 3 | 13 | 11 | ... | ... |
| 12-15 | 74 (180) | 85 | 4 | 3 | 9 | 8 | ... | ... |
| 16-20 | 73 (222) | 82 | 5 | ... | 9 | 3 | ... | ... |
| 21-30 | 264 (595) | 87 | 3 | 0.5 | 8.5 | 3 | ... | ... |
| 31 and upwards | 263 (726) | 74 | 1 | 1 | 5 | 2 | ... | ... |
| 0-15 | 363 (954) | 80 | 8 | 4 | 15 | 10 | 4 | 0.7 |
| 16 and upwards | 601 (1543) | 80 | 2.6 | 0.8 | 6 | 2 | 0.4 | 0.3 |

* The number in brackets indicates the number of spleen examinations; the other figure the number of blood and spleen examinations.

TABLE II.

Shewing the number of infections of simple tertian, quartan, subtertian (trophozoites and crescents) associated with the various degrees of splenic enlargement.

| Degree of splenic enlargement | SIMPLE TERTIAN | | QUARTAN | | SUBTERTIAN | | | |
|----------------------------------|-------------------------|---|--------------------|---|--------------------|--|--------------------|--|
| | | | | | Trophozoites | | Crescents | |
| | Number infected . | Percentage of all tertian infections | Number infected | Percentage of all quartan infections | Number infected | Percentage of all tropho- zoite infections | Number infected | Percentage of all crescent infections |
| No enlargement ... | 64 | 21 | 7 | 11 | 118 | 17 | 88 | 15 |
| I and II* ... | 125 | 41 | 24 | 37 | 286 | 41 | 253 | 42 |
| III and IV* ... | 97 | 32 | 28 | 43 | 255 | 37 | 227 | 38 |
| V* and more ... | 16 | 6 | 6 | 9 | 33 | 5 | 27 | 5 |
| Average enlargement | | 2.5 | | 2.8 | | 2.6 | | 2.5 |

* For explanation of these figures, *vide* Table XV.

TABLE III.

Shewing the number of crescents in relation to the number of trophozoites parasites.

| Number of cases | Number of trophozoites | NUMBER OF CRESCENT CASES | | | | Total | Percent- age ^a |
|-----------------|------------------------------------|---------------------------------|------------------------------|--|------------------------------|-------|------------------------------|
| | | 1 crescent per 1-100 leucocytes | | 1 crescent per 101 and more leucocytes | | | |
| | | Total | Percent- age ^a | Total | Percent- age ^a | | |
| 207 | 100 and more per 50 leucocytes | 66 | 8.4 | 41 | 5.2 | 107 | 13.6 |
| 517 | 1-500 per 500 leucocytes ... | 136 | 17.3 | 104 | 13.3 | 240 | 30.6 |
| 621 | 1 and less per 3000 leucocytes ... | 206 | 25.7 | 231 | 30.1 | 437 | 55.8 |

* i.e., Percentage of the total number of crescent cases found (784).

TABLE IV.

Blood and spleen examinations in Tandjong-Pinang and Penjengat (Riouw Archipelago).

Population : Tandjong-Pinang, 660; Penjengat, 895

(cf. Chart No. 3).

| | Age in years | Number examined* | Spleen rate | INFECTION RATE | | | | $\frac{c}{t}$ |
|-----------------|----------------|------------------|-------------|----------------|---------|-------------|-----------|---------------|
| | | | | Simple tertian | Quartan | Sub-tertian | Crescents | |
| Tandjong-Pinang | 0-15 | 113 (174) | 68 | 11 | 12 | 18 | 7 | 0.4 |
| " | 16 and upwards | 96 (124) | 73 | 10 | 5 | 9 | 1 | 0.1 |
| Penjengat | 0-15 | 66 (129) | 69 | 9 | 24 | 4 | ... | ... |
| " | 16 and upwards | 31 (190) | 30 | 13 | 6 | ... | ... | ... |

* The number in brackets indicates the number of spleen examinations; the other figure, the number of blood and spleen examinations.

TABLE V.

Blood and spleen examinations in Tegal. Population. A, 6184; B, 2653.

(cf. Chart No. 4).

| Age in years | Number examined* | Spleen rate | INFECTION RATE | | | | | | $\frac{c}{t}$ |
|-----------------|---------------------|----------------|-------------------|---------|-----------------|-----------|-----------|-----|---------------|
| | | | Simple Tertian | Quartan | Sub- tertian | Crescents | | | |
| | | | | | | Total | Infective | | |
| A† { | 0-15 | 260 (374) | 62 | 4 | 1.5 | 15 | 6.5 | 3 | 0.4 |
| | 16 and upwards | 244 (657) | 65 | ... | 0.4 | 9.4 | 0.8 | ... | 0.09 |
| B† { | 0-15 | 131 (167) | 45 | 5 | ... | 22 | 11 | 6 | 0.5 |
| | 16 and upwards | 107 (196) | 54 | 2 | ... | 14 | 7 | 4 | 0.5 |

* The number in brackets indicates the number of spleen examinations, the other figure the number of blood and spleen examinations.

†A : An endemic region. †B : A sub-endemic region with epidemic exacerbations.

TABLE VI.

Blood and spleen examinations in Semarang III. Population 13,855.
(cf. Chart No. 5).

| Age in years | Number examined* | Spleen rate | INFECTION RATE. | | | |
|-------------------|---------------------|----------------|-------------------|---------|------------|-----------|
| | | | Simple tertian | Quartan | Subtertian | Crescents |
| 0-3 | 56 (198) | 34 | 7 | ... | 2 | ... |
| 4-5 | 52 (144) | 51 | 11 | 2 | 6 | 4 |
| 6-7 | 108 (274) | 40 | 4 | ... | 7 | 3 |
| 8-11 | 134 (352) | 40 | 3 | 1 | 5 | 2 |
| 12-15 | 69 (182) | 44 | 1.5 | ... | 1.5 | 1 |
| 16-20 | 60 (220) | 52 | 1.5 | 1.5 | 7 | 7 |
| 21-30 | 201 (567) | 45 | 0.5 | 0.5 | 2 | 1 |
| 31 and upwards | 248 (637) | 41 | 0.4 | 0.4 | 2 | 1 |

* The number in brackets indicates the number of spleen examinations, the other figure, the number of blood and spleen examinations.

TABLE VII.

Blood and spleen examinations in Semarang IV. Population 36,978.
(cf. Chart No. 6).

| Age in years | Number examined* | Spleen rate | INFECTION RATE. | | | |
|-------------------|---------------------|----------------|-------------------|---------|------------|-----------|
| | | | Simple tertian | Quartan | Subtertian | Crescents |
| 0-3 | 67 (384) | 11 | ... | ... | 1 | 1 |
| 4-5 | 66 (286) | 17 | 1 | ... | 3 | 3 |
| 6-7 | 232 (790) | 20 | 2.5 | 1 | 2.5 | 2 |
| 8-11 | 187 (854) | 21 | 2 | ... | 1 | 0.5 |
| 12-15 | 125 (527) | 19 | 1 | ... | 1 | 1 |
| 16-20 | 70 (498) | 21 | ... | ... | ... | ... |
| 21-30 | 211 (1014) | 20 | 0.5 | ... | 0.5 | 0.4 |
| 31 and upwards | 196 (1271) | 19 | 0.5 | 0.5 | ... | ... |

* The number in brackets indicates the number of spleen examinations, the other figure, the number of blood and spleen examinations.

TABLE VIII.

Blood and spleen examinations in North Soendatar. Population 1,264.
(cf Chart No. 7)

| Age in years | Number examined | Spleen rate | INFECTION RATE | | | | $\frac{c}{t}$ |
|-------------------|--------------------|----------------|-------------------|---------|------------|-----------|---------------|
| | | | Simple tertian | Quartan | Subtertian | Crescents | |
| 0-5 | 129 | 81 | 26 | 12 | 65 | 46 | 0.7 |
| 6-9 | 100 | 96 | 22 | 4 | 77 | 54 | |
| 10-15 | 69 | 87 | 12 | 13 | 59 | 43 | |
| 16-20 | 86 | 93 | 13 | 5 | 78 | 50 | 0.6 |
| 21-30 | 95 | 84 | 16 | 3 | 54 | 40 | |
| 31-and upwards | 139 | 85 | 6 | 4 | 37 | 21 | |

TABLE IX.

Blood and spleen examinations in South Soendatar. Population 1,031.
(cf. Chart No. 8).

| Age in years | Number examined | Spleen rate | INFECTION RATE | | | | $\frac{c}{t}$ |
|-------------------|--------------------|----------------|-------------------|---------|------------|-----------|---------------|
| | | | Simple Tertian | Quartan | Subtertian | Crescents | |
| 0-5 | 113 | 24 | 4 | 2 | 18 | 11 | 0.7 |
| 6-9 | 75 | 21 | 4 | 1 | 19 | 16 | |
| 10-15 | 37 | 20 | 3 | 3 | 13 | 13 | |
| 16-20 | 77 | 30 | 8 | 1 | 25 | 18 | 0.7 |
| 21-30 | 59 | 29 | 2 | 7 | 13 | 7 | |
| 31 and upwards | 87 | 30 | 2 | 1 | 16 | 14 | |

TABLE X.

Blood and spleen examinations in Modjodjedjer. Population 640.
(cf. Chart No. 9).

| Age in years | Number examined | Spleen rate | INFECTION RATE | | | | $\frac{c}{t}$ |
|-------------------|--------------------|----------------|-------------------|---------|------------|-----------|---------------|
| | | | Simple Tertian | Quartan | Subtertian | Crescents | |
| 0-5 | 97 | 32 | 12 | ... | 18 | 6 | 0.4 |
| 6-9 | 129 | 37 | 19 | ... | 24 | 12 | |
| 10-15 | 73 | 40 | 9 | ... | 41 | 18 | |
| 16-20 | 79 | 39 | 9 | ... | 38 | 19 | 0.4 |
| 21-30 | 88 | 34 | 10 | ... | 24 | 9 | |
| 31 and upwards | 90 | 30 | 3 | ... | 22 | 15 | |

TABLE XI.

Blood and spleen examinations before and after quininisation in North Soendatar.
(cf. Charts Nos. 10 and 14).

| Age in years | Number examined | Spleen rate | INFECTION RATE. | | | | | c t | Percentage of parasite cases without enlarged spleen |
|-----------------------|--------------------|----------------|-------------------|---------|-----------------|-----------|-----------|--------|---|
| | | | Simple tertian | Quartan | Sub- tertian | Crescents | | | |
| | | | | | | Total | Infective | | |
| Before the treatment. | | | | | | | | | |
| 0-15 | 298 | 90 | 21 | 9 | 68 | 48 | 28 | 0.7 | 0.9 |
| 16 and upwards | 320 | 87 | 11 | 4 | 53 | 34 | 18 | 0.6 | 2 |
| After the treatment. | | | | | | | | | |
| 0-15 | 235 | 90 | 10 | 0.4 | 62 | 41 | 20 | 0.7 | 6 |
| 16 and upwards | 196 | 73 | 4 | ... | 39 | 21 | 9 | 0.5 | 26 |

TABLE XI.

Results of quinisation of a definite group of individuals in North Soendatar examined before and after a month's treatment.
(cf. Chart No. 10a).

| Age in years | Number examined | INFECTION RATE | | | | | $\frac{c}{t}$ | Spleen rate | Average splenic enlarge- ment | Average reduction of enlarge- ment | Percentage of parasite cases without enlarged spleen |
|--------------------|--------------------|-------------------|---------|-----------------|-----------|-----------|---------------|----------------|--|--|---|
| | | Simple tertian | Quartan | Sub- tertian | Crescents | | | | | | |
| | | | | | Total | Infective | | | | | |
| Before the 0-15 | treatment 105 | 25 | 10 | 79 | 56 | 34 | 0.7 | 86 | 3.1 | ... | 6 |
| 16 and upwards | 105 | 8 | 1 | 63 | 48 | 25 | 0.8 | 89 | 2.9 | 26 % | 8 |
| After the 0-15 | treatment 90 | 17 | 3 | 71 | 50 | 34 | 0.7 | 84 | 2.3 | ... | 12 |
| 16 and upwards | 90 | 4 | ... | 49 | 29 | 10 | 0.6 | 65 | 2.1 | 27 % | 29 |

TABLE XII.

Blood and spleen examinations, before and after quinisation in South Soendatar.
(cf. Charts Nos. 11 and 14).

| Age in years | Number examined | Spleen rate | INFECTION RATE | | | | | $\frac{c}{t}$ | Percentage of parasite cases without enlarged spleen |
|-----------------------|--------------------|----------------|-------------------|---------|-----------------|-----------|-----------|---------------|---|
| | | | Simple tertian | Quartan | Sub- tertian | Crescents | | | |
| | | | | | | Total | Infective | | |
| Before the treatment. | | | | | | | | | |
| 0-15 | 225 | 21 | 4 | 2 | 18 | 13 | 9 | 0.7 | 28 |
| 16 and upwards | 223 | 29 | 4 | 3 | 18 | 13 | 9 | 0.7 | 28 |
| After the treatment. | | | | | | | | | |
| 0-15 | 94 | 40 | 6 | 1 | 30 | 18 | 9 | 0.6 | 33 |
| 16 and upwards | 102 | 30 | 1 | 1 | 27 | 8 | 2 | 0.3 | 78 |

TABLE XIII.

Blood and spleen examinations in Modjodjedjer

A At the onset of the epidemic (June).

B During house to house quininisation (July).

C After three months quininisation (September).
(cf. Charts Nos. 11, 14 and 15).

| Time | Age in years | Number examined | Spleen rate | INFECTION RATE | | | | | $\frac{c}{t}$ | Percentage of parasite cases without enlarged spleen |
|-----------|----------------|-----------------|-------------|----------------|---------|-------------|-----------|-----------|---------------|--|
| | | | | Simple tertian | Quartan | Sub-tertian | Crescents | | | |
| | | | | | | | Total | Infective | | |
| June ... | 0-15 | 197 | 37 | 14 | ... | 25 | 10 | 3 | 0.4 | 22 |
| | 16 and upwards | 184 | 32 | 9 | ... | 26 | 13 | 5 | 0.5 | 34 (43-26)* |
| July ... | 0-15 | 102 | 34 | 13 | ... | 29 | 14 | 11 | 0.5 | 46 |
| | 16 and upwards | 72 | 39 | 7 | ... | 32 | 18 | 9 | 0.6 | 46 (54-40)* |
| Sept. ... | 0-15 | 138 | 59 | 19 | 3 | 22 | 16 | 8 | 0.7 | 9 |
| | 16 and upwards | 113 | 26 | 0.9 | 6 | 11 | 2.6 | 0.8 | 0.2 | 55 (77-4)* |

* The figures in brackets give the percentage, mentioned at the head of the column, for men and women separately. The first figure refers to men and the second to women.

TABLE XIV.

Blood and spleen examinations of the school-children of Margaredjo compared with those of children of similar age in Semarang I-III

(cf. Chart Nos. 13 and 14).

| Age in years | Number examined* | Spleen rate | INFECTION RATE | | | | | $\frac{c}{t}$ | Percentage of parasite cases without enlarged spleen |
|-----------------|---------------------|----------------|-------------------|---------|-----------------|-----------|-----------|---------------|---|
| | | | Simple Tertian | Quartan | Sub- tertian | Crescents | | | |
| | | | | | | Total | Infective | | |
| MARGAREDO | | | | | | | | | |
| 6-9 | 105 | 64 | 6 | 1 | 22 | 15 | 9 | 0.7 | 7 |
| 10-11 | 40 | 45 | 2.5 | ... | 22.5 | 2.5 | ... | 0.1 | 39 |
| 12-13 | 59 | 32 | ... | ... | 13 | ... | ... | ... | |
| SEMARANG I-III. | | | | | | | | | |
| 6-7 | 208 | 58 | 5 | 3 | 10 | 4 | 1 | 0.4 | 8 |
| 8-11 | 245 | 59 | 4 | 2 | 8 | 6 | 3 | 0.7 | |
| 12-15 | 143 | 64 | 3 | 1 | 5 | 5 | 3 | 1.0 | |

* All the school children were examined.

TABLE XV.

Splenic enlargement before and after quinine treatment.

| Locality | Before or after the treatment | Children or Adults | Classes of enlargement* | | | | | | | | Average of † enlargement | Average reduction | |
|---------------------|-------------------------------|--------------------|-------------------------|----|----|-----|----|-----|-----|-----|--------------------------|-------------------|--------|
| | | | o | I | II | III | IV | V | VI | VII | | | VIII |
| SOUTH SOENDATAR ... | before | children | 178 | 24 | 7 | 9 | 7 | ... | ... | ... | ... | 2.0 | ... |
| | after | adults | 157 | 35 | 14 | 6 | 10 | 1 | ... | ... | ... | 1.9 | ... |
| | | children | 56 | 17 | 14 | 5 | 2 | ... | ... | ... | ... | 1.8 | + 10 % |
| | | adults | 71 | 23 | 6 | ... | 2 | ... | ... | ... | ... | 1.4 | + 26 % |
| NORTH SOENDATAR ... | before | children | 34 | 38 | 60 | 60 | 92 | 27 | 4 | ... | ... | 3.1 | ... |
| | after | adults | 45 | 60 | 51 | 82 | 81 | 22 | 9 | ... | ... | 2.9 | ... |
| | | children | 24 | 66 | 56 | 44 | 38 | 7 | ... | ... | ... | 2.3 | + 26 % |
| | | adults | 53 | 62 | 34 | 28 | 15 | 4 | ... | ... | ... | 2.0 | + 31 % |
| MORJOYUDJER ... | before | children | 192 | 72 | 19 | 11 | 5 | ... | ... | ... | ... | 1.5 | ... |
| | after | adults | 174 | 67 | 14 | 4 | 2 | ... | ... | ... | ... | 1.3 | ... |
| | | children | 36 | 19 | 10 | 8 | 10 | ... | ... | ... | ... | 1.8 | - 20 % |
| | | adults | 28 | 6 | 2 | 1 | 3 | ... | ... | ... | ... | 2.1 | - 61 % |
| MARGAREDO ... | before | children | 38 | 28 | 15 | 14 | 9 | 1 | ... | ... | ... | 2.1 | ... |
| | after | children | 62 | 20 | 5 | 8 | 2 | 2 | ... | ... | ... | 1.9 | + 9 % |

* The degree of enlargement is determined as follows:—Draw a line from the angle of the 9th rib, through the umbilicus, to the right anterior superior spine of the ilium. This line has three fixed points: the umbilicus and the points of junction with the rib and the ilium. The portions of the line above and below the umbilicus are divided into four equal parts, by plotting out on the line nine equidistant points, numbered 1-9, No. 1 being the junction with the rib, No. 5 the umbilicus and No. 9 the junction with the ilium. Now we call (Schuffner and Swellengrebel, 1918):

A spleen just palpable reaching point No. 2: splenic enlargement I.

A spleen passing point No. 2, reaching point No. 3: splenic enlargement II.

A spleen passing point No. 4, reaching the umbilicus: splenic enlargement IV., etc.

† To determine this average, to spleens of Class I is ascribed a value 1, to spleens of Class II a value 2, etc.

e.g. The average of enlargement of the spleens of the children of South Soendatar is:—

$$\frac{(24 \times 1) + (7 \times 2) + (9 \times 3) + (7 \times 4)}{24 + 7 + 9 + 7}$$

$$\text{i.e., } \frac{93}{47} = 1.97 = 2.$$

ROSS (1910) and others have described similar methods for expressing in a simple way the degree of splenic enlargement.

TABLE XVI.

Number of crescents in the districts under observation.

| Locality | Number of gamete cases observed | Number of cases where one crescent was found per | | | | | Percentage of highly infective cases | Children or Adults |
|--|---------------------------------|--|-------|--------|---------|-------|--------------------------------------|--------------------|
| | | 10-20 | 21-50 | 51-100 | 101-200 | 201 + | | |
| | | leucocytes | | | | | | |
| SEMARANG I, II ... | 43 | 5 | 8 | 5 | 2 | 23 | 42 | children |
| | 19 | 2 | 1 | 1 | ... | 15 | 20 | adults |
| SEMARANG III—V ... | 17 | 1 | 4 | 2 | ... | 10 | 41 | children |
| | 9 | 3 | 2 | 2 | ... | 2 | 77 | adults |
| TEGAL— Endemic ... | 17 | 5 | 2 | 1 | ... | 9 | 46 | children |
| | 2 | ... | ... | ... | ... | 2 | ... | adults |
| Sub-endemic ... | 15 | 5 | 2 | 1 | ... | 7 | 53 | children |
| | 7 | 1 | 3 | ... | ... | 3 | 57 | adults |
| MONJODJEDJER— Before quininisation | 33 | 6 | 8 | 3 | 1 | 15 | 51 | children |
| | 36 | 6 | 7 | 2 | 2 | 19 | 42 | adults |
| After quininisation | 22 | 2 | 5 | 4 | 1 | 10 | 50 | children |
| | 3 | ... | 1 | ... | ... | 2 | 33 | adults |
| SOUTH SOENDATAR— Before quininisation | 29 | 6 | 7 | 6 | ... | 10 | 66 | children |
| | 33 | 6 | 11 | 7 | ... | 9 | 72 | adults |
| After quininisation | 17 | 4 | 3 | 2 | 1 | 7 | 52 | children |
| | 8 | 1 | 1 | ... | 1 | 5 | 25 | adults |
| NORTH SOENDATAR— Before quininisation | 143 | 43 | 31 | 9 | 13 | 47 | 58 | children |
| | 110 | 21 | 23 | 16 | 4 | 46 | 54 | adults |
| After quininisation | 97 | 13 | 28 | 7 | 7 | 42 | 49 | children |
| | 43 | 5 | 12 | 2 | 4 | 20 | 44 | adults |
| MARGAREDO— 6-9 years of age ... | 16 | 5 | 4 | 1 | ... | 6 | 62 | children |
| 10-13 years of age ... | 1 | ... | ... | ... | ... | 1 | ... | children |

TABLE XVII

Showing spleen rate of children and adults in some of the islands of the Australian division of the Malay Archipelago.

| Locality | CHILDREN | | ADULTS | |
|--|-----------------|-------------|-----------------|-------------|
| | Number examined | Spleen rate | Number examined | Spleen rate |
| Ternate ^a | 121 | 78 % | 44 | 70 % |
| Gilolo | 19 | 100 % | 22 | 82 % |
| Small islands between Halmaheira and Amboina | 65 | 71 % | 41 | 73 % |
| Amboina* | 276 | 93 % | 184 | 87 % |
| Ceram | 126 | 93 % | 56 | 84 % |
| Western New Guinea | 25 | 72 % | 32 | 84 % |
| Total | 632 | 87 % | 379 | 83 % |

^a Highly infected part of the island.

TABLE XVIII.

Showing percentage of mixed infections among the parasite cases.

| Locality | CHILDREN | | | ADULTS | | |
|--------------------------|---------------------------------|----------------------------------|-----------------|--------------------------------|----------------------------------|-----------------|
| | Number of parasite cases. | Number of mixed infections | Per- centage | Number of parasite cases | Number of mixed infections | Per- centage |
| MODJODJEDJER— | | | | | | |
| Before quininisation ... | 127 | 8 | 6 | 93 | 3 | 3 |
| After quininisation ... | 53 | 5 | 9 | 21 | 1 | 5 |
| SOUTH SOENDATAR | | | | | | |
| Before quininisation ... | 43 | 9 | 21 | 51 | 7 | 14 |
| After quininisation ... | 34 | 4 | 12 | 27 | 1 | 4 |
| MARGAREDO— | | | | | | |
| Before quininisation ... | 29 | ... | ... | ... | ... | .. |
| After quininisation ... | 18 | ... | ... | ... | ... | ... |
| TEGAL— | | | | | | |
| Endemic | 50 | 4 | 8 | 25 | ... | ... |
| Sub-endemic | 34 | 2 | 6 | 15 | 2 | 13 |
| SEMARANG— | | | | | | |
| I-II | 91 | 8 | 9 | 67 | 2 | 3 |
| III | 38 | ... | ... | 18 | 1 | 5 |
| IV | 26 | ... | ... | 6 | ... | ... |
| RIOUW— | | | | | | |
| T. pinang | 47 | 4 | 8 | 22 | 3 | 14 |
| Penjengat | 24 | 1 | 4 | 6 | ... | ... |
| NORTH SOENDATAR— | | | | | | |
| Before quininisation ... | 230 | 56 | 24 | 181 | 30 | 16 |
| After quininisation ... | 154 | 16 | 10 | 85 | 2 | 2 |

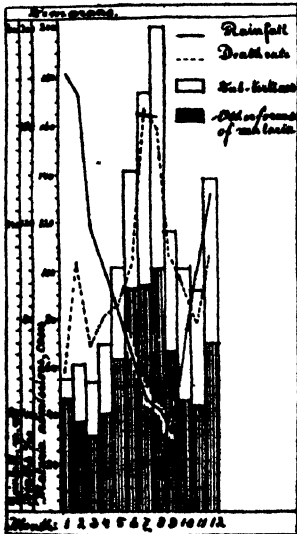
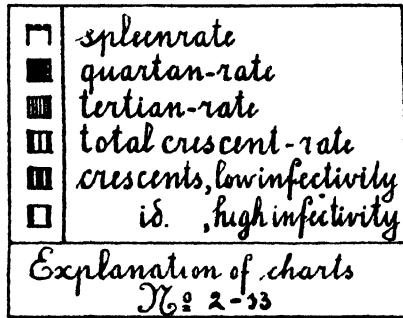


CHART 1. Malaria admissions to the native hospital, rainfall and death-rates in Semarang.

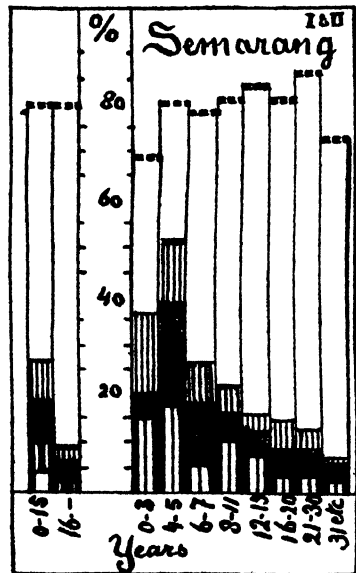


CHART 2. Littoral districts of Semarang, endemic area. Blood and spleen examinations in children and adults (left), arranged according to the age (right).

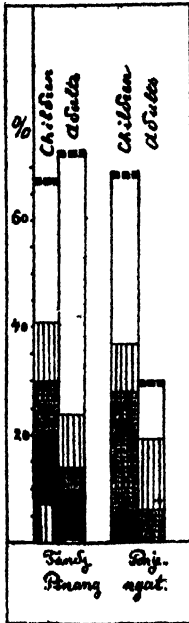


CHART 3. Aberrant results of blood and spleen examinations in an endemic region (Riouw Archipelago: Tandjong Pinang and Penjengat)

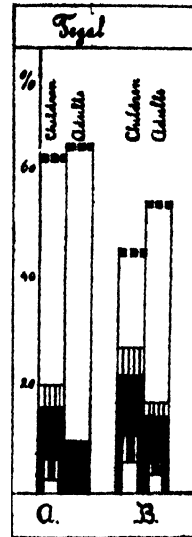
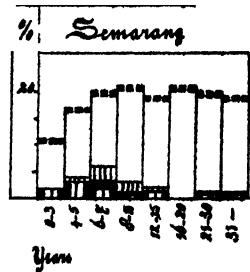
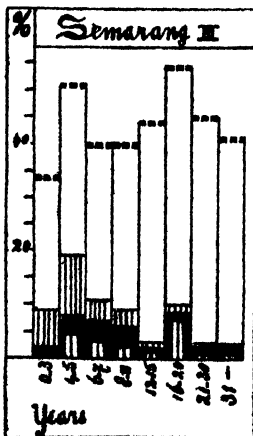


CHART 4. Blood and spleen examinations in children and adults in endemic region and adjoining sub-endemic region, showing epidemic exacerbation.



CHARTS 5 and 6. Blood and spleen examinations in sub-endemic regions arranged according to the age (Semarang).

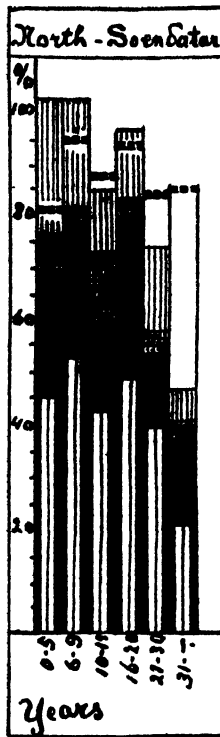
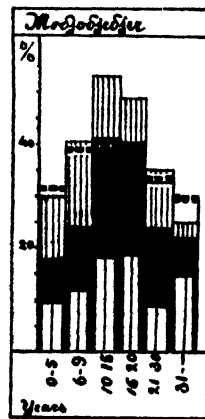
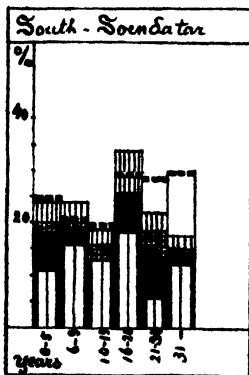


CHART 7 Blood and spleen examinations in epidemic region of high endemicity, arranged according to the age (North Soendatar)



CHARTS 8 and 9. Ibid. in epidemic region of lower malarial frequency (South Soendatar and Modjodjedjer).

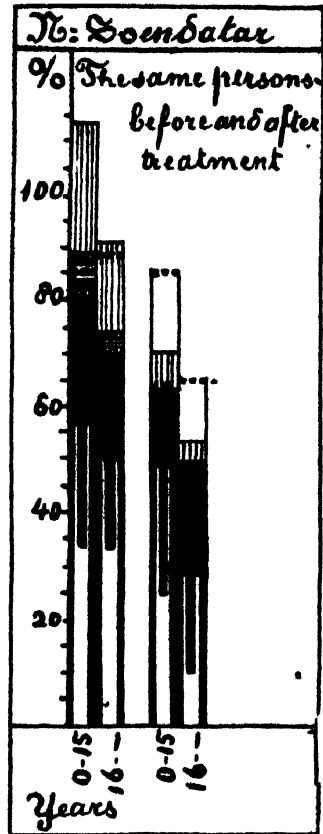
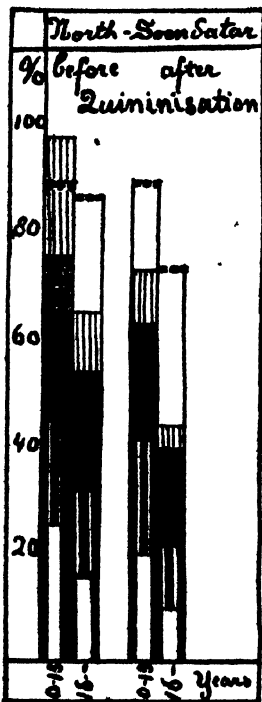


CHART 10. Blood and spleen examinations in North Soendatar, before and after quinine treatment. CHART 10A gives the result of similar examinations of 195 individuals examined before and after treatment.

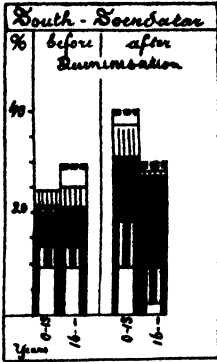


CHART 11. Ibid. in South Soendatar.

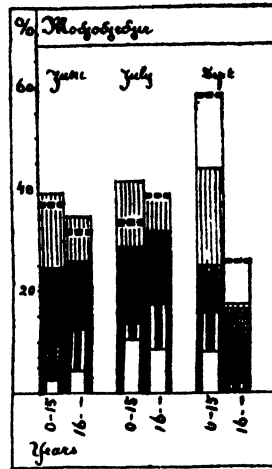


CHART 12. Blood and spleen examinations in Modjodjedjer: at the beginning of the epidemic (June), during the house-to-house quinine distribution (July) and after two additional months of treatment at the clinic.

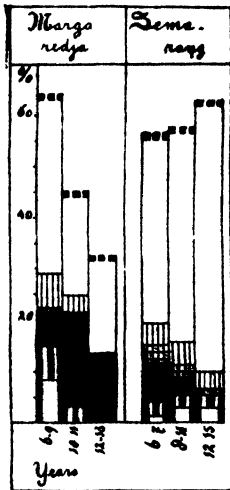


CHART 13. Blood and spleen examinations of school-children in Margaredjo, arranged in classes according to their subjection to the treatment for a longer or shorter period. For comparison: Children in Semarang I-III of the same age.

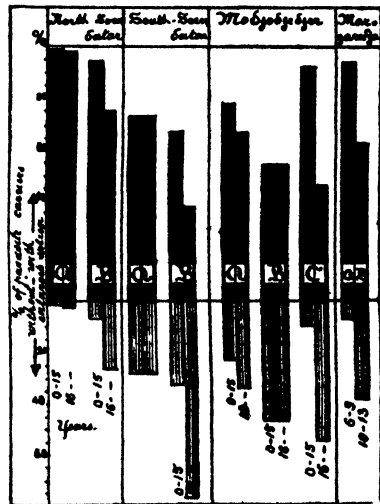


CHART 14. Percentage of parasite cases without enlarged spleen.

- North Soendatar { A. Before treatment.
South Soendatar { B. After treatment.
Modjodjedjer { A. Beginning of the epidemic.
B. House-to-house quinine distribution.
C. After two additional months of treatment at the clinic.

HEAT AND *STEGOMYIA FASCIATA*: SHORT EXPOSURES TO RAISED TEMPERATURES

BY

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(Received for publication 6 May, 1920)

Early in the course of these experiments on the effects of heat on *Stegomyia fasciata* it was found that the results were materially influenced by three factors, namely, (1) the manner in which the heat was applied, (2) the length of time occupied in reaching the particular temperature, and (3) the length of time the temperature was maintained. In the first instance, therefore, the effects of exposure for a short period (five minutes) were studied; the results observed are recorded in the following pages. It is hoped to investigate the effects of longer exposures as soon as the necessary apparatus can be procured.

THE EFFECTS OF EXPOSURE FOR A SHORT PERIOD TO AN INCREASE OF TEMPERATURE

EGGS

The eggs used in the first experiments (Nos. 1 to 8) had been laid on filter-paper, and had been preserved dry for a fortnight. Small pieces of the filter-paper, each with ten eggs attached, were cut off and put dry into glass tubes; they were then flooded with water at the required temperature, maintained at that temperature for five minutes, and then allowed to cool at the laboratory temperature (28°C.). A control was put up for each experiment, the eggs being flooded with water at the temperature of the air in the laboratory. Only unshrunk eggs were used. Finally a loop-full of faeces was added to each tube. The tubes were watched for one week.

The effects on the eggs of immersion in this manner for five minutes in water heated to various temperatures are summarised in Table I.

TABLE I.

Experiments on dried eggs immersed in water.

| Experiment No. | Temperature | Number of eggs used | Number of larvae which hatched | Remarks | CONTROL TUBES | | |
|----------------|-------------|---------------------|--------------------------------|---|---------------------|--------------------------------|-----------------------------------|
| | | | | | Number of eggs used | Number of larvae which hatched | Remarks. |
| 1 | °C. 49 | 10 | ... | Eggs did not uncap. | 10 | 10 | |
| 2 | 48 | 10 | 1 | Hatched on the third day | 10 | 8 | Only seven left on the sixth day. |
| 3 | 47 | 10 | 2 | One hatched on the first day, and one on the third. | 10 | 10 | |
| 4 | 46 | 10 | 8 | | 10 | 10 | |
| 5 | 45 | 10 | 10 | Only nine left on the seventh day. | 10 | 8 | |
| 6 | 44 | 10 | 9 | | 10 | 10 | Only five left on the sixth day. |
| 7 | 43 | 10 | 10 | | 10 | 9 | |
| 8 | 42 | 10 | 10 | Only seven left on the sixth day. | 10 | 10 | Only six left on the sixth day. |

The eggs appeared to withstand successfully temperatures as high as 46° C.; they were generally killed at temperatures higher than this, and none hatched of those which had been submitted to a temperature of 40° C.

Somewhat similar experiments were carried out with recently laid eggs, that is with eggs which had been laid less than forty-eight hours and which had not been allowed to dry. The eggs had been deposited on wet filter-paper about twenty-four hours before the experiments were started. Pieces of the filter-paper with eggs attached were exposed to different temperatures, the technique employed being similar to that in the experiments with adults (see p. 7) excepting that sufficient water was present in the vessels to keep the eggs moist. After five minutes' exposure to the particular temperature to be tested, the slip of filter-paper was flooded with water at the temperature of the air in the laboratory.

The results of two such experiments, combined and summarised in Table II, were similar to those with dried eggs.

TABLE II.

Experiments on recently laid eggs.

| Experiment No. | Temperature | Number of eggs used | Number of larvae which hatched | CONTROLS | |
|----------------|-------------|---------------------|--------------------------------|---------------------|--------------------------------|
| | | | | Number of eggs used | Number of larvae which hatched |
| 9 | °C. 49 | 20 | 0 | 15 | 11 |
| 10 | 47 | 20 | 4 | 15 | 9 |
| 11 | 45 | 44 | 33 | 40 | 27 |

Eggs were also subjected to various temperatures in a dry condition; the technique employed being similar to that used in the experiments with adults (see p. 7). The eggs had been laid on filter-paper, and had been preserved dry for two months. Immediately after exposure the eggs were flooded with water at the temperature of the air in the laboratory. Only unshrunk eggs were used. The results are summarised in Table III.

TABLE III.

Experiments on eggs in a dry condition.

| Experiment No. | Temperature | Number of eggs used | Number of larvae which hatched | CONTROLS | |
|----------------|-------------|---------------------|--------------------------------|---------------------|---------------------------------|
| | | | | Number of eggs used | Number of larvae which hatched. |
| 12 | °C. 49 | 10 | 0 | 10 | 10 |
| 13 | 47 | 10 | 1 | 7 | 6 |
| 14 | 45 | 5 | 4 | 6 | 6 |

The effects of exposure for five minutes to various temperatures were similar on (1) recently laid eggs which had not been dried, and on (2) dried eggs either with or without water: a temperature of 49° C. was fatal to all, and a temperature of 47° C. to most of the eggs used; a temperature of 45° C. had no appreciable ill-effect.

LARVAE

These experiments were conducted in a water-bath, kept at a constant temperature, in which there floated a small and thin-walled glass vessel or beaker. The larvae for an experiment were placed in the beaker and freed from water by means of a pipette. The beaker was then immersed in the water-bath and half filled with the heated water. Care was taken to arrange that the beaker should float in the water-bath and that the surface of the water in it should be lower than that in the water-bath. The temperature was read on a thermometer held in the beaker.

The experiments lasted five minutes each. At the end of this time the beaker was lifted out of the water-bath and allowed to cool at the temperature of the air in the laboratory (27° to 28° C.).

The larvae used were in the final stage of their development. The effects upon them of immersion for five minutes in water heated to various temperatures are summarised in Table IV.

TABLE IV.
Experiments on larvae.

| Experiment No. | Temperature | Number of larvae used in the experiment | State after five minutes' exposure. | Final result |
|----------------|--------------|---|---|---|
| 15 | 50° | 6 | All inert | None revived. |
| 16 | $47-48$ | 6 | All inert | None revived |
| 17 | $46-47$ | 6 | All inert | None revived. |
| 18 | 46 | 10 | All inert | Two revived partially, but died within one day. |
| 19 | 45 | 6 | All inert | Two revived partially, but died within one day. |
| 20 | 44 | 10 | All inert | Seven revived partially; four of these died within one day, and three pupated but died soon afterwards with the larval pelt still attached to their fins. |
| 21 | 43 | 10 | None inert, but all profoundly affected | Five died as larvae, one died pupating, four pupated and hatched. The mosquitoes produced fertile eggs. |
| 22 | 42 | 10 | None inert, but all considerably affected | Five died as larvae, one died pupating, four pupated and hatched. The mosquitoes produced fertile eggs. |
| 23 | 41 | 10 | All alive, but affected slightly | All revived, pupated, and hatched. The mosquitoes produced fertile eggs. |
| 24 | 40 | 6 | All alive and active | All pupated and hatched. The mosquitoes produced fertile eggs. |

Immediate results. After five minutes' immersion in water heated to 44° C. or higher the larvae were all inert, and generally lay either at the bottom of the beaker or flat on the surface of the water; at slightly lower temperatures (41° to 43° C.) the effect was similar but slighter, the larvae being sluggish or showing signs of life only when disturbed; temperatures of 40° C. and under did not have any apparent deleterious effect.

Final results. Larvae rendered inert by immersion in heated water sometimes recovered after being removed from the water-bath. In some cases the recovery was only temporary or partial; in other cases it was complete, that is, the larvae became normally active and subsequently completed their development. In a few cases the larvae recovered completely to all appearances, but when they pupated they were unable to free themselves from the larval pelt which adhered to their paddles and interfered with their movements to such a degree that they died in a short time.

In the experiments summarised in Table IV the larvae seldom survived temperatures above 43° C., and suffered no permanent ill-effects from temperatures under 42° C.; no recovery was observed after exposure to temperatures of 47° C. or higher, and no complete recovery after exposure to temperatures above 43° C.

PUPAE

The experiments with pupae were carried out in the same manner as those with larvae. The effects on the pupae of immersion for five minutes in water heated to various temperatures are summarised in Table V.

Immediate results. After five minutes' immersion in water heated to 44° C. or higher the pupae were all inert and lying at the surface; the higher the temperature the more rapidly the pupae were effected in this manner. At slightly lower temperatures (42° to 43° C.) the effect was similar but slighter, the pupae being sluggish and having a tendency to remain at the surface, or showing signs of life only when disturbed. Temperatures of 41° C. and under did not have any apparent deleterious effect.

Final results. Pupae rendered inert by immersion in heated water sometimes recovered after being removed from the water-bath. The recovery was in some cases only temporary or partial; in others

it was complete, that is, the pupae became normally active and subsequently completed their development. In some cases the pupae appeared to have recovered completely, but the adult insects were unable to hatch from them or emerged imperfectly and so died,

TABLE V.
Experiments on pupae.

| Experiment No. | Temperature | Number of pupae used in the experiment | State after five minutes' exposure | Final result. |
|----------------|-------------|--|---------------------------------------|---|
| 25 | °C. 50 | 6 | All inert | None revived. |
| 26 | 47-48 | 6 | All inert | Three revived partially, but died within two days. |
| 27 | 46-47 | 5 | All inert | Two revived partially, but died within two days. |
| 28 | 46 | 10 | All inert | Eight revived; of these seven died within two days (three or more in the act of hatching), and one hatched, but the mosquito was not able to free itself completely from the pupal pelt. |
| 29 | 45 | 6 | All inert | Five revived; of these one died hatching, and four hatched. |
| 30 | 44 | 10 | All inert | Nine revived; of these one died within one day, one died hatching, and seven hatched. Five of the seven mosquitoes which hatched appeared to be more or less paralysed. One male and one female were isolated; fertile eggs were produced |
| 31 | 43 | 10 | None inert, but all affected | All revived and hatched; the mosquitoes produced fertile eggs. |
| 32 | 42 | 10 | None inert, but all somewhat affected | Nine revived and hatched; one mosquito was unable to free its hind legs from the pupal pelt. Five males and two females, which appeared normal, were isolated; fertile eggs were produced. |
| 33 | 41 | 10 | All alive and active | All hatched; the mosquitoes produced fertile eggs. |
| 34 | 40 | 6 | All alive and active | All hatched; the mosquitoes produced fertile eggs. |

most commonly with the thorax protruded, or outstretched at the surface having failed to split the pupal case. Sometimes the mosquitoes withdrew completely, all but the ends of their legs. Not a few of the mosquitoes hatched from the pupae used in these

experiments appeared to be partially paralysed, their power of flight being particularly affected.

In the experiments summarised in Table V the pupae seldom survived temperatures above $45^{\circ}\text{C}.$, and seldom suffered permanent ill-effects from temperatures under $44^{\circ}\text{C}.$; no recovery was observed in the pupae subjected to a temperature of $50^{\circ}\text{C}.$, and no complete recovery after exposure to temperatures above $45^{\circ}\text{C}.$

The immediate effects of immersion in heated water on the larvae and pupae of *Stegomyia fasciata* are similar, both being rendered inert by subjection to a temperature of $44^{\circ}\text{C}.$ or higher. The larvae when reduced to this state usually either sink or float parallel with the surface of the water; the pupae float at the surface. The final effects differ somewhat. Pupae appear to be more resistant than larvae, and recover more frequently and after subjection to higher temperatures. The heat, however, appears to have some action on the pupal case, making it more difficult to split and thus increasing the dangers incidental to eclosion. A corresponding effect was observed in only a few larvae, and was shown by the adherence of the larval pelt to the paddles of the pupa.

ADULTS

The experiments with adults were conducted in a small glass flask immersed in a water-bath kept at a constant temperature. At the bottom of the flask was a drop of water and a layer of lint or wool, across the middle a paper loop for the mosquitoes to alight on, and in the neck a plug of wool through which passed a thermometer. The flask was immersed up to the neck in the water-bath to a level about the middle of the woollen plug, and was held in position until the thermometer in it registered the required temperature. The plug was then withdrawn for a moment and the mosquitoes introduced enclosed in a small gauze bag folded in such a way that it opened immediately it was released. In each experiment the temperature was maintained for five minutes, after which the flask was removed and allowed to cool at the temperature of the air in the laboratory ($28^{\circ}\text{C}.$).

The effects on the mosquitoes of exposure in this manner to various temperatures for five minutes are summarised in Table VI.

Immediate results. The mosquitoes exposed to temperatures of 41° C. or higher were all rendered inert; those exposed to 40° C. were profoundly affected but were not completely inactivated, and those exposed to 39° C. remained fairly active. At the higher temperatures the effect appeared to be almost instantaneous.

Final results. Mosquitoes rendered inert by exposure to high temperatures sometimes recovered after being removed from the

TABLE VI.
Experiments on Adults.

| Experiment No. | Temperature | Number of mosquitoes used in the experiment | | State after five minutes' exposure | Final result |
|----------------|-------------|---|--------|------------------------------------|---|
| | | Male | Female | | |
| | °C. | | | | |
| 35 | 47 | 1 | 1 | Inert | Did not revive. |
| 36 | 46 | 3 | 6 | Inert | Did not revive |
| 37 | 45 | 2 | 2 | Inert | Did not revive. |
| 38 | 44 | 2 | 1 | Inert | Did not revive. |
| 39 | 43 | 1 | 7 | Inert | Revived slowly, but partly paralysed. All but three females died within three days. |
| 40 | 42 | 1 | 1 | Inert | Revived slowly, but female partly paralysed; fertile eggs produced. |
| 41 | 41 | 2 | 3 | Inert | Revived. |
| 42 | 40 | 2 | 3 | Profoundly affected | Revived. |
| 43 | 39 | 1 | 3 | All more or less active | |

water-bath, but the recovery was slow. In the experiments summarised in Table VI no recovery took place after exposure to temperatures of 44° C. or higher. After exposure to 43° C. the eight mosquitoes used in the experiment revived slowly: after twenty-four hours all eight were still alive but only four of them had revived sufficiently to be able to crawl about, and none could fly; after three days, three still survived, and of them only one eventually regained its usual activity. The wings appeared to be particularly affected, for mosquitoes which revived partially were usually able to walk but unable to fly, and others less affected sometimes were

unable to fold back the wings but held them permanently at right angles to the long axis of the body. The female mosquito in Experiment No. 40 had the right wing affected in this way, she was nevertheless able to fly, to suck blood, and to lay fertile eggs.

The mosquitoes used in the foregoing experiments had not been fed in any way. In order to determine if they were more susceptible to the action of heat in this state than after a meal fourteen mosquitoes (five males and nine females), which had previously been provided with honey-water, were exposed to a temperature of 44°C . in the manner already described. All were rendered inert, and showed no signs of life two hours after removal from the water-bath. Fifteen hours later two females were just alive, one with the right wing, and one with both wings outstretched and paralysed. Two days later both these mosquitoes had died without recovering further. The results of these experiments were, therefore, similar to those with fasting mosquitoes.

A trace of moisture was present in the flask in the foregoing experiments. One additional experiment was, therefore, made without this. Five mosquitoes (two males and three females) were exposed to a temperature of 41°C . for five minutes in the manner described, but in a dry flask. All were rendered inert. Subsequently all but one revived completely, but rather more slowly than in the previous experiment at this temperature (No. 41). One mosquito, a male, revived only partially; both his wings were paralysed and he survived only four days. The absence of moisture, therefore, appeared to have the effect of making the mosquitoes slightly more susceptible to the action of a raised temperature.

TABLE VII.
Comparison of effects.

| Stage of development | Highest temperature survived by half or more without permanent injury | Lowest temperature survived by none without permanent injury |
|----------------------|---|--|
| | $^{\circ}\text{C}$. | $^{\circ}\text{C}$. |
| Eggs | 46 | 49 |
| Larvae | 41 | 44 |
| Pupae | 43 | 46 |
| Adults | 42 | 44 |

CONCLUSIONS

The ability of *Stegomyia fasciata* to withstand sudden exposure for five minutes to a raised temperature is greatest in the egg stage, slightly less in the pupal stage, and least in the larval and adult stages (see Table VII).

I.—ORAL ADMINISTRATION OF QUININE OR QUININE AND ARSENIC FOR SHORT PERIODS TO YOUNG NATIVE CHILDREN INFECTED WITH MALIGNANT TERTIAN MALARIA

BY

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AND

M. W. FRASER

(Received for publication 6 May, 1920)

The observations recorded in this paper were made at Accra, in the Gold Coast, West Africa, during the months of July and August, 1919.

All the patients were native children infected in the Gold Coast. In every instance a diagnosis of malignant tertian malaria was made microscopically, and in all cases trophozoites were present in the blood on the day treatment was begun. Except when otherwise indicated in the tables, blood examinations were made daily until the parasites disappeared from the cutaneous blood, and thereafter once a week during an after-treatment observation period of two months. In Tables I and IV, in the Remarks columns, the days referred to are days of the after-treatment observation period.

All the children, excepting No. 17, appeared to be healthy; some of them, however, had recently had fever, and all were heavily infected with malignant tertian malaria, the parasites being either numerous or very numerous in the blood. All excepting Nos. 3, 5, 7, 9, 12, 15, 16, 20 and 21 were boys.

The children were not admitted to hospital, but both during treatment and observation continued to live in their own homes and to go about as usual. For this reason temperature charts were not kept, but the parents were instructed to bring the children to the dispensary at once should they appear to be unwell. In the tables, therefore, a febrile lapse indicates a clinical attack of fever as complained of by the patient or detected by the parents.

It was not possible to guard against the possibility of reinfection, so that the observations might be expected to show if anything an undue preponderance of malaria relapses. As a matter of fact, Anopheline mosquitoes were very uncommon during the period covered by the observations, and six negative cases selected as controls did not become infected.

The quinine was administered by the mouth, in solution.

The following sets of observations were made:—

GROUP A

Quinine hydrochloride grains 10 orally were given in eight cases (Nos. 1 to 8), the treatment being continued for two consecutive days only in five cases, and for three, seven, and nine days respectively in the remaining three cases. The ages of the children varied from six months to seven years. The results of these treatments are recorded in Table I.

TABLE I.

Summary of results of oral administration of quinine hydrochloride grains 10 to native children in malignant tertian malaria.

| Number of case | Age in years | Date of end of treatment | Number of days on which treatment was given | Trophozoites disappeared from cutaneous blood in — days after first dose | Trophozoite relapse occurred in — days after last dose | Febrile relapse occurred in — days after last dose | Remarks |
|----------------|--------------|--------------------------|---|--|--|--|---|
| 1 | 6/12 | 30.7.19 | 2 | 2 | 8-14 | ... | No febrile relapse in eight weeks. |
| 2 | 3 | 31.7.19 | 2 | 1 | 7-14 | ... | No febrile relapse in five weeks. |
| 3 | 4 | 9.8.19 | 2 | Present throughout | ... | ... | No fever. Quinine and arsenic administered on the 3rd day. |
| 4 | 5 | 31.7.19 | 2 | Present throughout | ... | ... | No fever. Quinine and arsenic administered on the 12th day. |
| 5 | 7 | 30.7.19 | 2 | 2 | 8-14 | ... | No febrile relapse in eight weeks. |
| 6 | 9/12 | 8.8.19 | 3 | Present throughout | ... | ... | No fever. Quinine and arsenic administered on the 4th day. |
| 7 | 2 | 6.8.19 | 7 | Present throughout | ... | ... | Fever on the 6th day: quinine administered. |
| 8 | 7 12 | 9.8.19 | 9 | Present throughout | ... | ... | No fever. Quinine and arsenic administered on the 3rd day. |

In one case (No. 8) the temperature fell to normal in one day; in the remaining seven cases treatment was commenced during an apyrexial period; three cases (Nos. 3, 6 and 7) had had fever the previous day.

In five cases trophozoites were present throughout, in the remaining three cases they disappeared from the cutaneous blood within two days. None of the patients had attacks of fever during the treatments.

Relapses. In five cases trophozoites did not disappear from the cutaneous blood; all remained positive at least a week, notwithstanding the fact that in some treatment had been restarted before the seventh day. In the three remaining cases, those which became negative, trophozoites reappeared in the blood in from seven to fourteen days, but no febrile relapse occurred in from five to eight weeks.

In these cases quinine hydrochloride grains 10 orally for periods ranging from two to nine consecutive days either failed to cause the disappearance of trophozoites from the blood, or failed to prevent their reappearance within a few days. The fact that owing to the variation in the ages of the patients the relative doses were different does not appear to have affected the result.

GROUP B

Quinine sulphate grains 10 orally were given in four cases (Nos. 9 to 12); the treatment being continued for four, six, twelve, and thirteen consecutive days respectively. In another case (No. 13) quinine sulphate grains $7\frac{1}{2}$ orally were given for fifteen days. The ages of the children varied from four months to four years. The results of these treatments are recorded in Table II.

In three of the cases treatment was begun during an apyrexial period, but all three had had fever the previous day. In the remaining two cases the temperature fell to normal in one day.

In one case (No. 12) trophozoites were present throughout; on the fourteenth day the treatment was abandoned. In the remaining four cases trophozoites disappeared from the cutaneous blood, in two cases in two days, in one case in ten or eleven days, and in one case the examinations were too infrequent to give an exact figure. None of the patients had attacks of fever during the treatments,

Relapses. In one case trophozoites did not disappear from the cutaneous blood. One of the remaining four cases did not relapse in an after-treatment observation period of seventy-two days. In the other three cases trophozoites reappeared in the blood within.

TABLE II.

Summary of results of oral administration of quinine sulphate grains 10 to native children in malignant tertian malaria.

| Number of case | Age in years | Date of end of treatment | Number of days on which treatment was given | Temperature fell to normal in — days after first dose | Trophozoites disappeared from cutaneous blood in — days after first dose | Trophozoite relapse occurred in — days after last dose | Febrile relapse occurred in — days after last dose | Observation period (in days) in case which did not relapse | Remarks |
|----------------|--------------|--------------------------|---|---|--|--|--|--|--|
| 9 | 4/12 | 14.7.19 | 4 | Apyrexia | 2 | ... | ... | 72 | |
| 10 | 3 | 18.8.19 | 6 | Apyrexia | 2 | 3-12 | 15 | ... | |
| 11 | 3 | 29.7.19 | 12 | Apyrexia | 10-11 | 9-15 | 14 | ... | |
| 12 | 2 | 30.7.19 | 13 | 1 | Present throughout | ... | ... | ... | No fever. Treatment changed after 13 days. |
| 13* | 4 | 8.7.19 | 15 | 1 | 1-9 | 11-10 | ... | ... | Quinine administered on the 11th day. |

* The dose in this case was 7½ grains.

fifteen days, and a febrile relapse occurred about the same time in two of them, the third case being again treated with quinine as soon as parasites reappeared.

The action of quinine sulphate in these cases appeared to be similar to the action of quinine hydrochloride in Group A.

GROUP C

Quinine sulphate grains 20 orally were given in four cases (Nos. 14 to 17); the treatment being continued for five, nine, fifteen, and seventeen consecutive days respectively. The ages of the children varied from seven months to three years. The results of these treatments are recorded in Table III.

In all the cases treatment was begun during an apyrexial period. In one case (No. 17) trophozoites were present throughout; the

treatment was abandoned on the eighteenth day. In the remaining three cases trophozoites disappeared from the cutaneous blood in two, six or seven, and twelve days respectively. None of the patients had attacks of fever during the treatments.

Relapses. In one case trophozoites did not disappear from the cutaneous blood. In the three remaining cases trophozoites

TABLE III.

Summary of results of oral administration of quinine sulphate grains 20 to native children in malignant tertian malaria.

| Number of case | Age in years | Date of end of treatment | Number of days on which treatment was given | Trophozoites disappeared from cutaneous blood in — days after first dose | Trophozoite relapse occurred in — days after last dose | Febrile relapse occurred in — days after last dose | Remarks |
|----------------|--------------|--------------------------|---|--|--|--|--|
| 14 | 3 | 4.7.19 | 5 | 2 | 6-12 | 11 | |
| 15 | 2 | 8.7.19 | 9 | 6-7 | 1-8 | 9 | |
| 16 | 3 | 14.7.19 | 15 | 12 | 3-9 | ... | No febrile relapse in a 72 days' after-treatment observation period. Parasites persisted. |
| 17 | 7/12 | 16.7.19 | 17 | Present throughout | ... | ... | No fever. After 17 days an intramuscular injection of quinine bihydrochloride grains 3 was given, blood negative next day; relapses, parasitic on 8-13th day, febrile on 13th day after injection. |

reappeared in the blood within twelve days; two of these cases had febrile relapses, on the ninth and eleventh days respectively, and one did not have a febrile relapse within an after-treatment observation period of seventy-two days.

It was our intention in these cases to give the quinine sulphate daily until the trophozoites disappeared from the blood, but as in the previous groups we found that in some patients quinine seemed to have little or no action on the parasites. Case No. 17 is remarkable in this respect. This child, only seven months old, took 20 grains of quinine daily for seventeen days, and, although the general condition of the child improved, at the end of this time the

parasites in the blood were as numerous as at the beginning. No vomiting and no other ill-effects were caused by the treatment. How much of the quinine was absorbed in this case, and in other similar cases, we do not know, but the parasites disappeared from the cutaneous blood within twenty-four hours after an intra-muscular injection of 3 grains of quinine bihydrochloride.

GROUP D

A mixture containing quinine hydrochloride grains 10 and liquor arsenici hydrochloridi minims 5 was given daily in five cases (Nos. 18 to 22), half the dose being given in the morning and half in the evening. In four of the cases the treatment was given for eight days; in the remaining case for only seven, because on the first day quinine sulphate grains 10 had been given in error. All the five children had been previously treated with quinine alone (see Table I), and had failed to go negative. The ages of the children varied from seven months to five years. The results of this treatment are recorded in Table IV.

TABLE IV.

Summary of results of oral administration of quinine and arsenic to native children in malignant tertian malaria

| Number of case | Age in years | Date of end of treatment | Number of days on which treatment was given | Trophozoites disappeared from cutaneous blood in — days after first dose | Trophozoite relapse occurred in — days after last dose | Febrile relapse occurred in — days after last dose | After-treatment observation period (in days) | Remarks |
|----------------|--------------|--------------------------|---|--|--|--|--|--|
| 18 | 7/12 | 20.8.19 | 8 | Present throughout | ... | ... | 35 | No fever. |
| 19 | 9/12 | 20.8.19 | 8 | 4 | ... | ... | 15 | Lost sight of after the 15th day |
| 20 | 2 | 20.8.19 | 7* | Present throughout | ... | 29 | 35 | A single febrile attack on the 29th day. |
| 21 | 4 | 20.8.19 | 8 | 5 | 11-15 | ... | 35 | No febrile relapse in five weeks |
| 22 | 5 | 20.8.19 | 8 | 3 | 22-28 | ... | 35 | No febrile relapse in five weeks |

* Because on the first day quinine sulphate 10 grains was given by mistake.

"In all the cases treatment was begun during an apyrexial period : in one case (No. 20) there had been fever on the previous day, but in the other four there had been no recent attacks of fever.

In two cases trophozoites were present throughout; in the remaining three cases trophozoites disappeared from the cutaneous blood in three, four, and five days respectively.

None of the patients experienced fever during treatment.

Relapses. In two cases trophozoites did not disappear from the cutaneous blood; both cases continued to show parasites to the end of the after-treatment observation period, which in this group was only thirty-five days; one case had no febrile relapse, the other (No. 20) had a single febrile attack, on the twenty-ninth day. One case (No. 19) was lost sight of fifteen days after the cessation of treatment: up to this time neither parasitic nor febrile relapse had occurred. In the remaining two cases trophozoites reappeared in the blood within a month; neither case had a febrile relapse within an after-treatment observation period of thirty-five days.

Quinine and arsenic treatment in the doses given, although it effected the disappearance of the parasites from the blood in three out of five cases which had previously resisted quinine alone, cannot be said to have had a more beneficial action than the treatments given in the earlier groups.

GROUP E (CONTROL)

It was not possible to exclude the chance of reinfection in the cases under observation after treatment. As a matter of fact, no rain fell during the period occupied by this investigation, and all mosquitoes, but especially Anophelines, were uncommon.

As a control we treated with quinine six children (Nos. 23 to 28), all under one year old, in whose blood malaria parasites were not found. Each child received quinine hydrochloride grains 10 orally for two consecutive days, the treatments being given between the 28th and 31st of July.

These children and those comprised in Groups A, B, C, and D lived in the same locality and under similar conditions, and were examined in the same manner.

During an observation period of two months none of these six children showed evidence of malarial infection.

The results of the Malaria Investigation carried out at the Liverpool School of Tropical Medicine from 1917 to 1919 showed that, in simple tertian malaria, the percentage of relapses were similar after a large number of different short quinine treatments. A similar series of observations on malignant tertian malaria was not made, but it was found that in this disease after intra-muscular injections of quinine bihydrochloride grains 15 on each of two consecutive days only all the cases relapsed (1919, A), and that no better results followed the same treatment when reinforced by an intravenous injection of novarsenobillon, or extended into a course of liquor arsenicalis minimis 30 by the mouth for sixteen days and quinine bihydrochloride grains 15 intra-muscularly on the first and second, eighth and ninth, fifteenth and sixteenth days (1919, B).

It may be permissible, therefore, to consider the seventeen cases included in Groups A, B, and C as forming a single series of malignant tertian malaria infections in young children treated by short courses of quinine. Of the seventeen cases, sixteen (equal to 94 per cent.) either did not become negative at all, or had a parasitic relapse. Of the nine cases which became negative and subsequently had a parasitic relapse four had also a febrile relapse, and two which did not relapse were observed for less than sixty days.

The results in the children who were under five years of age may be separately recorded for comparison with those in school boys from five to eighteen years of age presently to be considered. Fifteen of the children were under five years of age: of these fourteen (equal to 93 per cent.) either did not become negative at all (six), or had a parasitic relapse (eight). Of the eight cases which became negative and subsequently had a parasitic relapse four had also a febrile relapse, and two which did not relapse were observed for less than sixty days.

The following facts may also be briefly noted. The native children took quinine hydrochloride grains 10, and quinine sulphate grains 10 or 20 without ill-effects. These doses brought the temperature down to normal in those cases suffering from fever, and prevented febrile attacks occurring during treatment. It may be thought that the doses given were excessive, and liable to cause injurious effects, among which gastro-intestinal disturbance leading to non-absorption might be one. Children, however, are known to

stand well relatively large doses of quinine; and as has already been stated, no ill-effects were observed in these cases. It is hardly necessary to say that before starting observations on the action of such large doses we had tried smaller ones.

In seven of the seventeen cases parasites persisted in the blood notwithstanding treatment. This fact emphasises the absolute necessity of blood examinations as a control in rational treatment of malaria, for it can scarcely be questioned that it is the presence of the parasites, not the occurrence of fever, that is the actual and potential danger in malaria. We do not know to what extent the failure was due to failure to absorb the quinine, but we do not think that it would be practicable to treat very young children with intramuscular injections, and we are convinced that any treatment for malaria in such subjects must be oral if it is to be favourably received by the patients themselves, their parents, and their physicians.

One other point of some importance is the fact illustrated by several of our cases (for example Nos. 1, 2, 5, 16, 18, 21, 22), namely, that native children may have malaria parasites in the blood *abundantly* for many consecutive weeks without being troubled by febrile attacks.

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II.—ORAL ADMINISTRATION OF QUININE SULPHATE GRAINS 20 TO ADULT NATIVES INFECTED WITH MALIGNANT TERTIAN MALARIA

BY

J. W. S. MACFIE

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Quinine sulphate grains 20 orally were given to eleven adult native men (Nos. 29 to 39) infected with malignant tertian malaria. All the cases were infected in the Gold Coast. The observations were made at Accra during July, 1919, and were undertaken for comparison with observations being made at the same time on the effects of oral administration of quinine to young native children.

In every instance a diagnosis of malignant tertian malaria was made microscopically, and in all cases trophozoites were present in the blood on the day treatment was begun. Blood examinations of ordinary thin films were made daily until the parasites disappeared, and thereafter once a week during an after-treatment observation period of two months. In the table, in the Remarks column, the days referred to are days of the after-treatment observation period.

The patients were not admitted to hospital, and continued to go about as usual; eight of the cases (Nos. 30 to 37), however, were inmates of the Accra asylum, and so were under constant observation. Temperature charts were not kept, but the patients and their guardians were instructed to report at once any occurrence of illness or fever.

The quinine was administered by the mouth, in solution.

It was our intention to give this treatment daily until the trophozoites disappeared from the cutaneous blood: this was done in nine cases, seven receiving treatment on two consecutive days, and two on three days, but inadvertently two cases received treatment for longer periods, namely, five and six days respectively. The results of these treatments are recorded in the table.

In one case (No. 39) the temperature fell to normal in one day. In the other cases treatment was begun during an apyrexial period.

In all the cases trophozoites disappeared from the cutaneous blood in one to two days.

Relapses. No parasitic or febrile relapses were observed during the after-treatment observation period of sixty to sixty-four days. In one case (No. 33) the observation period was less than sixty days, namely, thirty-three days.

TABLE —

Summary of results of oral administration of quinine sulphate grains 20, to adult natives in Malignant Tertian Malaria.

| Number of case | Date of end of treatment | Number of consecutive days on which treatment was given | Trophozoites disappeared from cutaneous blood in — days after first dose | Trophozoite relapse occurred in — days after last dose | Febrile relapse occurred in — days after last dose | After-treatment observation period (in days) in cases which did not relapse | Remarks |
|----------------|--------------------------|---|--|--|--|---|--|
| 29 | 22.7.19 | 2 | 1 | ... | ... | 64 | Crescents found on the 1st, 6th and 18th days. |
| 30 | 25.7.19 | 2 | 1 | ... | ... | 61 | |
| 31 | 25.7.19 | 2 | 1 | ... | ... | 61 | |
| 32 | 25.7.19 | 2 | 1 | .. | ... | 61 | |
| 33 | 25.7.19 | 2 | 1 | .. | ... | 33 | |
| 34 | 25.7.19 | 2 | 1 | .. | . | 61 | |
| 35 | 25.7.19 | 2 | 1 | .. | ... | 61 | |
| 36 | 26.7.19 | 3 | 2 | ... | ... | 60 | Crescents found on the 60th day. |
| 37 | 26.7.19 | 3 | 2 | .. | ... | 60 | |
| 38 | 28.7.19 | 5 | 2 | .. | ... | 60 | |
| 39 | 10.7.19 | 6 | 1 | ... | ... | 62 | |

If it is permissible to group these eleven cases together as short treatments with quinine sulphate grains 20 of cases of malignant tertian malaria in adult natives, the percentage of parasitic relapses would be: minimum 0, maximum 9.

These results should be compared with those of Group C of the previous paper (see p. 86); they should also be compared with the results of the treatment of malignant tertian malaria with intramuscular injections of quinine, with or without the addition of arsenic, in Europeans in Liverpool (1919).

REFERENCE

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III.—ORAL ADMINISTRATION OF QUININE SULPHATE GRAINS 10 DAILY FOR TWO CONSECUTIVE DAYS ONLY TO NATIVE SCHOOL-BOYS INFECTED WITH MALIGNANT TERTIAN MALARIA

BY

J. W. S. MACFIE

(Received for publication 6 May, 1920)

The observations recorded in the two previous papers showed that there was a marked difference between native children and adults infected with malignant tertian malaria in their response to short quinine treatments. In the latter the parasites rapidly disappeared from the cutaneous blood, and neither parasitic nor febrile relapses were observed within an after-treatment observation period of two months. In the former the parasites frequently persisted in the cutaneous blood, and of those that became negative almost all had a parasitic relapse, and about one-half a febrile relapse also.

With a view to obtaining further information on this matter, permission was granted to examine a number of boys attending schools in Accra and to treat with quinine those found to be infected with malaria. All the boys who were thus examined and treated were volunteers. I have to thank Mr. J. Dewhurst, of the Gold Coast Education Department, for the interest he took in the investigation, without which it would not have been possible to carry out the observations, and Dr. M. W. Fraser for much assistance in collecting the blood films at the beginning of the work. But for the fact that other duties intervened, Dr. Fraser and I had hoped to carry out these and other observations on malaria together, and I deeply regret that our work came to an untimely end.

As in the two previous papers, all the observations were made at

Accra, in the Gold Coast, West Africa. They were started on the 9th September, 1919, and ended on the 19th December, 1919; the dates on which the treatments ended are given in the text.

All the subjects were native school boys infected in the Gold Coast. In every instance a diagnosis of malignant tertian malaria was made microscopically, and in all cases trophozoites were present in the blood on the day treatment was begun. Excepting when otherwise indicated in the tables, blood examinations were made daily until the parasites disappeared from the cutaneous blood, and thereafter once a week during an after-treatment observation period of two months, the final (ninth) examination being made on or immediately after the sixtieth day. In the tables, in the Remarks columns, the days referred to are days of the after-treatment observation period.

All the boys appeared to be healthy. The fact that they were infected with malaria was only discovered by blood examination. During the observations they lived at their own homes, continued to attend school, and went about as usual.

Temperature charts were not kept. The boys were questioned as to the occurrence of illness or fever. In the case of the older boys this method of obtaining information was probably satisfactory, but in the case of the young boys it was not. In order to obtain some idea of the amount of illness caused by the infections, and the number of relapses, resort was had to the daily attendance register at the schools. This evidence is of a rather negative character: that is, if the attendance was regular it may be assumed that the boy was not ill, but if irregular the conclusion is less certain because there were many causes of absence besides illness, and mere lateness of arrival was accounted as absence. Such as it is, however, this evidence is of some value, because the attendances were very regular and clearly indicated that little serious illness troubled the boys.

The ages of natives in West Africa are seldom accurately known. The school boys on whom observations were made were arranged by their teachers in four age-groups, as follows:—Group A, 15 to 18; Group B, 12 to 14; Group C, 9 to 11; and Group D, 5 to 8 years. The treatment given to each case was the same, namely, quinine sulphate grains 10 orally on each of two consecutive days only. The quinine was given in solution.

In the tables, which summarised the results observed, the following symbols are used :—

- = No malaria parasites found.
- t = Malignant tertian trophozoites found.
- c = Malignant tertian gametes (crescents) found.
- T** = Quartan trophozoites found.
- G** = Quartan gametes found.

As it was not possible to exclude the chance of reinfection of the cases under observation after treatment, a few boys in whose blood parasites were not found were used as controls. These boys were given the same dose of quinine, and were examined in exactly the same manner as the others.

GROUP A (ages 15 to 18 years)

* Eleven boys (Nos. 40 to 50) in this age-group were treated. The date of the end of treatment was the 11th of September, 1919. The eight weekly examinations were made on the seventh, fourteenth, twenty-first, twenty-ninth, thirty-fifth, forty-second, forty-ninth and fifty-sixth days after the cessation of treatment, and the final examination on the sixtieth day: occasionally, for unavoidable reasons, a blood examination had to be made a day or two late in one or two individuals. Six negative cases (Nos. 51 to 56) were used as controls: they received the same treatment and were examined on the same days as the other boys. The results are recorded in Table I.

In all the cases treatment was begun during an apyrexial period.

Trophozoites disappeared from the cutaneous blood either on the day treatment was begun or in one to two days.

Relapses. Seven of the eleven cases (64 per cent.) had parasitic relapses within the observation period. In them the parasites were found rather irregularly as shown in the table, and only one case (No. 47) admitted having had a febrile relapse. The regular attendance at school of the others, and their healthy appearance, was not suggestive of any considerable amount of illness, and the boys themselves stated that they had not suffered from fever.

None of the six control cases showed evidence of malaria during the observation period, and the attendance at school of all of them was regular throughout.

TABLE I.

Group A: School boys, 15 to 18 years.

| Number of case | Trophozoites disappeared from cutaneous blood in — days after first dose | Results of blood examinations | | | | | | | | | Trophozoite relapse occurred in — days after last dose | Febrile relapse reported in — days after last dose | Observation period (in days) | Attendance at school during the observation period and other remarks |
|----------------|--|-------------------------------|-----|-----|-----|-----|-----|-----|-----|-----|--|--|------------------------------|--|
| | | 1st | 2nd | 3rd | 4th | 5th | 6th | 7th | 8th | 9th | | | | |
| 40 | Same day | — | — | — | — | — | — | — | — | — | ... | ... | 60 | Attendance regular throughout. |
| 41 | 1 | — | — | — | t | t | t | — | — | — | 22-29 | No febrile relapse in 60 days | 60 | Attendance regular throughout |
| 42 | 2 | ... | t | — | t | t | — | — | — | t | 1-14 | No febrile relapse in 60 days | 60 | Absent on the 6th, 7th and 8th days owing to toothache; otherwise attendance regular |
| 43 | 1 | — | — | — | — | — | — | — | — | — | ... | ... | 60 | Attendance regular throughout. |
| 44 | 1 | — | — | — | t | — | — | — | — | — | 22-29 | No febrile relapse in 60 days | 60 | Attendance regular throughout. |
| 45 | 1 | — | — | — | t | — | — | — | t | t | 22-29 | No febrile relapse in 60 days | 60 | Absent on the 39th day, owing to headache; otherwise attendance regular. |
| 46 | 1 | — | — | — | — | t | t | — | — | — | 30-35 | No febrile relapse in 60 days | 60 | Attendance regular throughout. |
| 47 | 2 | — | t | — | — | — | t | — | — | — | 8-14 | 15 | 60 | Absent on the 15th and 43rd days owing to fever; otherwise attendance regular. |
| 48 | 2 | — | — | — | — | — | — | t | — | — | 43-49 | No febrile relapse in 60 days | 60 | Absent on the 40th day, but not owing to illness; otherwise attendance regular. |
| 49 | 1 | — | — | — | — | — | — | — | — | — | ... | ... | 60 | Attendance regular throughout. |
| 50 | 1 | — | — | — | — | — | — | — | — | — | ... | ... | 63 | Attendance regular throughout. |
| 51 to 56 | Six control case. | — | — | — | — | — | — | — | — | — | ... | ... | 60 | Attendance of all six regular throughout. |

GROUP B (ages 12 to 14 years)

Nineteen boys (Nos. 57 to 75) in this age-group were treated. The date of the end of treatment was the 17th of September, 1919. The eight weekly examinations were made on the ninth, sixteenth, twenty-first, thirtieth, thirty-seventh, forty-fourth, fifty-first, and fifty-seventh days after the cessation of treatment, and the final examination on the sixty-first day: on one occasion, owing to absence on the proper day, an examination had to be made a day late. Two negative cases (Nos. 76 and 77) were used as controls: they received the same treatment and were examined in the same manner as the other boys. The results are recorded in Table II.

In all the cases treatment was begun during an apyrexial period.

Trophozoites disappeared from the cutaneous blood in from one to two days.

Relapses. Seventeen of the nineteen cases (89 per cent.) had parasitic relapses within the observation period. In them the parasites were found rather irregularly as shown in the table, and so far as could be ascertained, no single case had a febrile relapse. The regularity of attendance at school of most of the boys, and their healthy appearance, certainly did not suggest that much illness was caused by the malaria infections, and in fact none of the boys admitted having been ill.

Neither of the two control cases showed evidence of malaria during the period they were under observation.

GROUP C (ages 9 to 11 years)

Thirteen boys (Nos. 78 to 90) in this age-group were treated. The date of the end of treatment was the 23rd of September, 1919. The eight weekly examinations were made on the eighth, fifteenth, twenty-second, twenty-ninth, thirty-seventh, forty-fourth, fifty-first, and fifty-seventh days after the cessation of treatment, and the final examination on the sixty-second day. Ten negative cases (Nos. 91 to 100) were used as controls: they received the same treatment and were examined in the same manner as the other boys. The results are recorded in Table III.

In all the cases treatment was begun during an apyrexial period.

Trophozoites disappeared from the cutaneous blood in from one to two days.

TABLE II. Group B. School boys, 12 to 14 years.

| Number of case | Trophozoites disappeared from cutaneous blood in — days after first dose | Results of blood examinations | | | | | | | | | Trophozoite relapse occurred in — days after last dose | Febrile relapse reported in — days after last dose | Observation period (in days) | Attendance at school during the observation period and other remarks |
|----------------|--|-------------------------------|-----|-----|-----|-----|-----|-----|-----|-----|--|--|------------------------------|---|
| | | 1st | 2nd | 3rd | 4th | 5th | 6th | 7th | 8th | 9th | | | | |
| 57 | 1 | — | — | t | — | — | t | — | — | — | 17-21 | No febrile relapse in 61 days | 61 | Attendance regular throughout. |
| 58 | 1 | — | — | — | — | t | — | f | t | — | 31-37 | No febrile relapse in 61 days | 61 | Attendance regular throughout. |
| 59 | 1 | — | — | — | — | t | — | — | c | — | 31-37 | No febrile relapse in 61 days | 61 | Attendance regular throughout. |
| 60 | 1 | — | — | — | — | — | — | — | — | — | ... | ... | 61 | Attendance regular throughout. |
| 61 | 1 | — | — | t | t | t | — | — | — | — | 17-21 | No febrile relapse in 61 days | 61 | Attendance regular throughout. |
| 62 | 1 | — | — | — | — | — | t | — | c | ... | 38-44 | No febrile relapse in 57 days | 57 | Absent on part of the 20th, 40th, and 47th days. |
| 63 | 1 | — | — | — | ... | — | — | — | t | t | 52-57 | No febrile relapse in 61 days | 61 | Absent on the 15th, 30th and 41st days; and on part of the 26th, 29th, 36th, and 49th days. |
| 64 | 1 | — | — | — | t | — | — | — | — | t | 22-30 | No febrile relapse in 61 days | 61 | Absent on the 5th and 33rd days; and on part of the 37th day. |
| 65 | 2 | — | — | — | — | — | t | t | t | t | 38-44 | No febrile relapse in 61 days | 61 | Attendance regular throughout. |
| 66 | 1 | — | — | — | t | — | t | — | c | — | 22-30 | No febrile relapse in 61 days | 61 | Attendance regular throughout. |
| 67 | 2 | — | t | t | t | — | — | t | t | t | 10-16 | No febrile relapse in 61 days | 61 | Attendance regular throughout. |
| 68 | 2 | — | — | t | t | — | — | t | — | — | 17-21 | No febrile relapse in 61 days | 61 | Absent on part of the 19th, 36th, 40th and 43rd days. |
| 69 | 1 | — | — | — | — | — | — | — | t | t | 52-57 | No febrile relapse in 61 days | 61 | Attendance regular throughout. |
| 70 | 2 | — | — | t | t | — | t | — | — | c | 17-21 | No febrile relapse in 61 days | 61 | Attendance regular throughout. |
| 71 | 2 | — | — | t | — | t | — | — | — | — | 17-21 | No febrile relapse in 61 days | 61 | Absent on part of the 47th and 50th days. |
| 72 | 2 | — | — | — | — | — | — | — | t | — | 52-57 | No febrile relapse in 61 days | 61 | Attendance regular throughout. |
| 73 | 2 | — | — | t | — | t | t | t | — | — | 17-21 | No febrile relapse in 61 days | 61 | Attendance regular throughout. |
| 74 | 1 | — | — | — | — | t | t | — | t | t | 31-37 | No febrile relapse in 61 days | 61 | Attendance regular throughout. |
| 75 | 1 | — | — | — | — | — | — | — | — | — | ... | ... | 61 | Absent on part of the 58th day. |
| 76 | Control | — | — | — | — | — | — | — | — | — | ... | ... | 61 | Absent on the 26th, 33rd and 47th days. |
| 77 | Control | — | — | — | — | ... | — | — | — | — | ... | ... | 61 | Absent on the 43rd day; and on part of the 1st, 2nd, 8th, 13th, 15th, 23rd, 26th, 28th, 34th, 37th, 47th, 48th and 49th days. |

TABLE III.

Group C. School boys, 9 to 11 years.

| Number of case | Trophozoites disappeared from cutaneous blood in — days after first dose | Results of blood examinations | | | | | | | | | Trophozoite relapse occurred in — days after last dose | Febrile relapse reported in — days after last dose | Observation period (in days) | Attendance at school during the observation period and other remarks |
|----------------|--|-------------------------------|-----|-----|-----|-----|-----|-----|-----|-----|--|--|------------------------------|--|
| | | 1st | 2nd | 3rd | 4th | 5th | 6th | 7th | 8th | 9th | | | | |
| 78 | 2 | — | — | — | — | ... | — | — | — | — | ... | ... | 62 | Absent on the 28th day; and on the 35th, 37th and part of the 38th days, ill with 'stomach.' |
| 79 | 2 | — | — | — | — | t | — | — | — | — | 30-37 | No febrile relapse in 62 days | 62 | Absent on part of the 28th and 59th days. |
| 80 | 1 | — | — | — | t | — | — | — | — | — | 23-29 | No febrile relapse in 62 days | 62 | Absent on part of the 8th, 9th and 36th days. |
| 81 | 1 | — | — | — | t | — | t | — | — | — | 23-29 | No febrile relapse in 62 days | 62 | Attendance regular throughout. |
| 82 | 1 | — | — | — | t | — | t | — | — | — | 23-29 | No febrile relapse in 62 days | 62 | Attendance regular throughout. |
| 83 | 1 | — | — | — | — | — | — | — | — | t | 58-62 | No febrile relapse in 62 days | 62 | Absent on the 20th day; and on part of the 41st day. |
| 84 | 2 | — | — | — | — | t | t | c | — | — | 30-37 | No febrile relapse in 62 days | 62 | Attendance regular throughout. |
| 85 | 2 | — | ... | — | — | — | — | — | — | — | ... | ... | 62 | Absent on the 41st day; and on the 15th and part of the 14th and 17th days with 'headache.' |
| 86 | 1 | — | — | — | — | — | — | ... | ... | ... | ... | ... | 44 | Absent on the 24th, 31st, 36th, 38th, 42nd and 43rd days; and on part of the 7th, 15th, 16th, 23rd and 41st days. Left the school. |
| 87 | 1 | — | — | — | t | t | — | t | t | t | 23-29 | No febrile relapse in 62 days | 62 | Attendance regular throughout. |
| 88 | 1 | — | — | — | — | — | — | — | — | — | ... | ... | 62 | Attendance regular throughout. |
| 89 | 2 | — | — | — | t | — | — | — | t | t | 23-29 | No febrile relapse in 62 days | 62 | Attendance regular throughout. |

TABLE III—*continued*.

| Number of case | Trophozoites disappeared from cutaneous blood in — days after first dose | Results of blood examinations | | | | | | | | | Trophozoite relapse occurred in — days after last dose | Febrile relapse reported in — days after last dose | Observation period (in days) | Attendance at school during the observation period and other remarks |
|----------------|--|-------------------------------|-----|-----|-----|------|------|-----|-----|-----|--|--|------------------------------|--|
| | | 1st | 2nd | 3rd | 4th | 5th | 6th | 7th | 8th | 9th | | | | |
| 90 | I | — | — | — | — | — | — | — | — | — | ... | ... | 62 | Absent on the 23rd and 24th days attending court; and on part of the 17th, 28th, 36th and 41st days. |
| 91 | Control | — | — | — | — | — | — | t | t | — | 45-51 | No febrile relapse in 62 days | 62 | Absent on part of the 23rd and 30th days. |
| 92 | Control | — | — | — | — | — | — | — | — | — | ... | ... | 62 | Attendance regular throughout. |
| 93 | Control | — | — | — | — | — | — | — | — | — | ... | ... | 62 | Absent on the 13th day. |
| 94 | Control | — | — | — | — | — | — | — | — | — | ... | ... | 62 | Attendance regular throughout. |
| 95 | Control | — | — | — | — | — | — | — | — | — | ... | ... | 62 | Attendance regular throughout. |
| 96 | Control | — | — | — | — | — | — | — | — | — | ... | ... | 62 | Absent on the 13th and 14th days owing to a death in the family. |
| 97 | Control | — | — | — | — | — | — | — | — | — | ... | ... | 62 | Absent on the 10th day; and on part of the 6th, 36th and 56th days. |
| 98 | Control | — | — | — | — | — | — | — | — | — | ... | ... | 62 | Absent on the 35th and 41st days; and on part of the 50th day. |
| 99 | Control | — | — | — | — | t | t | t | — | — | 30-37 | No febrile relapse in 62 days | 62 | Attendance regular throughout. |
| 100 | Control | — | — | — | T | T.G. | T.G. | — | — | — | 23-29 | No febrile relapse in 62 days | 62 | Attendance regular throughout. |

Relapses. Seven of the thirteen cases had parasitic relapses within a sixty-day observation period; one was only found to have relapsed at the last examination, so that he may or may not have relapsed within sixty days; and one was observed for less than sixty days, namely, for forty-four days. The number of parasitic relapses within a sixty-day observation period may, therefore, have been from seven to nine, and the percentage from 54 to 69. In those cases which relapsed parasites were found with considerable irregularity, as is shown in the table. So far as could be ascertained, none of the cases had febrile relapses: illness was denied by the boys, and their attendance at school was on the whole regular, and certainly not less regular than the attendance of the boys who did not relapse.

Two of the ten control cases (20 per cent.) showed parasites of malignant tertian malaria, and one quartan parasites during the observation period. A proportion of the relapses referred to above may therefore have been reinfections, but it should be remembered that the examinations of relapse cases has shown that parasites may not be found in the cutaneous blood during several consecutive weeks.

GROUP D (ages 5 to 8 years)

Nineteen boys (Nos. 101 to 119) in this age-group were treated. The date of the end of treatment was the 28th of October, 1919. Owing to an unfortunate delay in obtaining permission to examine these small boys the Christmas holidays intervened before the expiration of the after-treatment observation period. Seven weekly examinations were, however, made, namely, on the eighth, fifteenth, twenty-second, twenty-ninth, thirty-sixth, forty-third, and fiftieth days after the cessation of treatment: on a few occasions, for unavoidable reasons, the blood examinations of one or two individuals had to be made a day late. Seven negative cases (Nos. 120 to 126) were used as controls: they received the same treatment and were examined in the same manner as the other boys. The results are recorded in Table IV.

In all the cases treatment was begun during an apyrexial period.

Trophozoites disappeared from the cutaneous blood in from one to two days.

Relapses. Twelve of the nineteen cases (63 per cent.) had

TABLE IV.

Group D. School boys, 5 to 8 years.

| Number of case | Trophozoites disappeared from cutaneous blood in — days after first dose | Results of blood examinations | | | | | | | Trophozoite relapse occurred in — days after last dose | Febrile relapse reported in — days after last dose | Observation period (in days) | Attendance at school during the observation period. Absent on part of the day at least on the following days :— |
|----------------|--|-------------------------------|-----|-----|-----|-----|-----|-----|--|--|------------------------------|---|
| | | 1st | 2nd | 3rd | 4th | 5th | 6th | 7th | | | | |
| 101 | 2 | — | — | — | — | — | — | — | ... | No febrile relapse reported. | 50 | 6th, 24th, 27th, 28th and 34th. |
| 102 | 1 | — | — | — | — | — | — | — | ... | | 50 | Attendance regular throughout. |
| 103 | 1 | — | — | — | — | t | — | — | 31-36 | | 50 | 8th, 9th and 22nd. |
| 104 | 1 | — | ... | — | — | t | t | — | 30-36 | | 50 | 6th, 15th, 34th, 35th and 43rd. |
| 105 | 2 | — | — | — | t | t | t | t | 23-29 | | 50 | 30th, 31st, 44th, 45th and 48th. |
| 106 | 1 | — | — | — | — | — | — | t | 44-50 | | 50 | 15th and 24th. |
| 107 | 1 | — | — | — | — | t | t | — | 30-36 | | 50 | 6th, 7th, 8th, 9th, 15th and 20th |
| 108 | 1 | — | — | t | t | t | t | — | 16-22 | | 50 | 6th, 7th, 8th, 9th, 22nd and 28th |
| 109 | 2 | — | — | — | — | — | — | — | ... | | 50 | 6th, 7th and 29th. |
| 110 | 1 | — | — | — | ... | — | t | t | 37-43 | | 50 | 10th, 34th and 35th. |
| 111 | 2 | — | — | — | — | — | — | — | ... | | 50 | 8th, 9th and 48th. |
| 112 | 1 | — | — | — | — | — | — | — | ... | | 51 | 6th and 22nd. |
| 113 | 2 | — | t | t | t | t | t | t | 9-15 | | 50 | Attendance regular throughout. |
| 114 | 2 | — | — | t | t | — | — | t | 16-22 | | 50 | 41st and 42nd. |
| 115 | 1 | — | — | — | t | ... | — | — | 23-29 | | 51 | 6th, 21st and 34th. |
| 116 | 1 | — | — | — | — | — | ... | — | ... | | 51 | 7th, 8th, 9th, 10th, 13th, 17th, 22nd, 23rd, 24th, 27th, 29th, 30th and 34th. |
| 117 | 2 | — | — | — | t | t | — | t | 23-29 | | 50 | 6th, 7th and 21st. |
| 118 | 1 | — | — | — | t | t | — | — | 23-30 | | 50 | 22nd and 34th. |
| 119 | 2 | — | — | — | — | ... | — | ... | ... | | 43 | 6th, 16th, 27th, 28th, 29th, 34th, 35th and 36th. |
| 120 | Control | — | — | — | t | t | — | — | 23-29 | No febrile relapse reported. | 50 | 14th and 27th. |
| 121 | Control | — | — | — | — | — | — | — | ... | | 50 | 6th. |
| 122 | Control | — | — | — | — | — | — | — | ... | | 50 | Attendance regular throughout. |
| 123 | Control | — | — | — | — | — | — | — | ... | | 50 | 6th. |
| 124 | Control | — | — | — | — | — | — | — | ... | | 50 | 6th and 7th. |
| 125 | Control | — | — | — | — | — | — | — | ... | | 50 | 6th, 22nd, 27th, 29th and 34th. |
| 126 | Control | — | — | — | — | — | — | — | ... | | 50 | 6th, 9th, 20th, 24th, 28th, 29th and 34th. |

parasitic relapses within the fifty-day observation period. In most of them the parasites were found irregularly, as is shown in the table. No reports of fever were received, but clearly very little reliance can be placed on the statements of young boys with regard to a matter such as the occurrence of febrile relapses. The attendance at school of such children was also not unnaturally rather irregular; there were 'epidemics' of absenteeism on certain days, and unpunctuality, with the penalty of being marked absent, was common. In the table are shown the days on which each child was marked absent during the observation period; the average numbers of absences were as follows: in cases which relapsed 3.9, in cases which did not relapse 2.6 days. This may be considered as indicating at any rate some degree of invalidity as the accompaniment of malaria infection. It will be noted, however, that the boy who was most frequently absent (No. 116) did not relapse, and that the boy who most consistently showed parasites in his blood (No. 113) was never absent.

One of the seven control cases (14 per cent.) showed malignant tertian malaria parasites during the observation period.

It should be repeated that the results in this Group are only comparable with those obtained in Groups A, B, and C up to the seventh weekly examination. In the latter Groups, taken together, twenty-eight out of the thirty-two relapses occurred during this part of the observation period. At the same rate the relapses in Group D during an observation period of sixty days should have equalled 72 per cent. This figure is a purely hypothetical one.

Cases which received half the treatment only

Eight boys (Nos. 127 to 134) belonging to the above age-groups received for one reason or another only half the standard treatment; that is, to each a single dose of quinine sulphate grains 10 was administered. The results of this abbreviated treatment are recorded in Table V

In all the cases treatment was begun during an apyrexial period.

In one case trophozoites did not disappear from the cutaneous blood; in six they disappeared in one to two days, and in one in two to nine days.

Relapses. In one case trophozoites did not disappear from the

TABLE V.

Cases which received half the treatment.

| Number of case | Group | Trophozoites disappeared from cutaneous blood in — days after first dose | Results of blood examinations | | | | | | | | | Trophozoite relapse occurred in — days after last dose | Febrile relapse reported in — days after last dose | Observation period (in days) |
|----------------|-------|--|-------------------------------|-----|-----|-----|-----|-----|-----|-----|-----|--|--|------------------------------|
| | | | 1st | 2nd | 3rd | 4th | 5th | 6th | 7th | 8th | 9th | | | |
| 127 | B | Present throughout | t | t | t | t | t | t | ... | t | t | Present throughout | ... | 62 |
| 128 | B | 2-9 | — | — | — | t | — | — | t | t | t | 22-30 | ... | 61 |
| 129 | B | 1-2 | — | — | ... | — | — | t | t | — | — | 39-45 | ... | 62 |
| 130 | B | 1-2 | — | — | — | ... | — | — | — | — | — | ... | ... | 62 |
| 131 | C | 1-2 | t | — | — | — | — | — | t | — | — | 3-9 | ... | 63 |
| 132 | C | 1-2 | — | — | t | t | — | — | — | — | — | 17-23 | ... | 64 |
| 133 | D | 1-2 | t | t | ... | ... | t | t | — | ... | . | 3-10 | ... | 51 |
| 134 | D | 1-2 | — | — | t | ... | ... | ... | ... | ... | . | 17-24 | ... | 24 |

cutaneous blood; in six of the remaining seven cases there was a parasitic relapse, in two as early as the first weekly examination. The treatment, therefore, either failed to cause the parasites to disappear or to prevent a relapse in seven out of eight cases (87.5 per cent.).

So far as could be ascertained, no febrile relapse occurred in any case.

SUMMARY AND CONCLUSION

Quinine sulphate grains 10 orally for two consecutive days only were given to sixty-two native school boys at Accra whose ages ranged from five to eighteen years. All the boys appeared to be healthy, and were found to be infected with malignant tertian malaria by blood examination.

This dose of quinine was sufficient in every case to cause the disappearance of the parasites from the cutaneous blood in one to two days.

After this treatment parasites reappeared in the blood in the majority of cases (see Table VI). The percentage of parasitic relapses was highest in the age-group comprising boys of twelve to fourteen years. In the absence of any other reason to account for this, it may be suggested that it was due to the critical period coincident with the advent of puberty.

TABLE VI.

| Age groups (years) | Number of cases | Percentage which relapsed parasitically within an after-treatment observation period of | |
|--------------------|-----------------|---|---------|
| | | 50 days | 60 days |
| 5-8 | 19 | 63 | [72] |
| 9-11 | 13 | 54-62 | 54-69 |
| 12-14 | 19 | 74 | 89 |
| 15-18 | 11 | 64 | 64 |

Only one of the sixty-two boys is definitely known to have had a febrile relapse; it is possible, however, that some of the youngest boys may have had malaria attacks that were not reported. From the regularity of attendance at school of the majority of the boys it is clear that the amount of illness accompanying the large number of parasitic relapses was very small.

Twenty-five boys in whose blood no parasites were found were used as controls, three showed malignant tertian parasites, and one quartan parasites during the observation periods.

These results should be compared with those following short quinine treatments administered to young native children and adult natives at Accra, and to adult Europeans at Liverpool. An exact comparison is not possible because the treatments were not identical, but it may be doubted if the differences were of a kind that would affect the incidence of parasitic and febrile relapses.

As regards the disappearance of the parasites from the blood, the native school boys responded to treatment in a manner similar to the adult natives and Europeans, but very different from the young native children.

The number of relapses, both parasitic and febrile, were less numerous than they were in the young native children or the adult Europeans, but more numerous than in the adult natives. The different results may, most naturally, be attributed to the development of a tolerance in the natives. It may at any rate be noted that the power of the native to cope with malaria infections begins to make itself felt early in life, has already attained a considerable degree of efficiency by the age of five to eight years, thereafter is maintained during adolescence with a remission at the age of puberty, and is enhanced in adult life.

The percentages of relapses, both parasitic and febrile, among the adult Europeans treated at Liverpool exceeded those among even young native children treated at Accra. This may have been due to an absence of or failure to develop tolerance on the part of the Europeans at Liverpool. It is possible, however, that another factor should be considered, namely, that malaria does not manifest itself in quite the same way in a temperate and a tropical climate.

In the course of the Liverpool Malaria Investigation, 1917-1919, it was found that, 'broadly speaking, a very small percentage of cures is obtained in the winter and spring and a comparatively high percentage in the summer and autumn,' and a graph was published showing that 'the higher the mean daily temperature the higher the percentage of cures.' At Liverpool the highest percentages of cures* were recorded during the months of July and August, when the mean daily temperature was about 60° F. In the tropics the mean daily temperature is high throughout the year, and it might, therefore, be anticipated that the percentage of cures would also be high. In Table VII are summarised the meteorological data recorded at Accra in the year 1919. If this factor is of importance its effects appear to be least during the first few years of life.

Unfortunately no corresponding series of observations could be carried out on Europeans at Accra. In a single case, infected between the 22nd and 27th of August, a malaria attack developed on the 6th of September, and malignant tertian parasites were found in the blood. Quinine hydrochloride grains 10 orally were taken.

* The term 'cure' in this connexion signifies no parasitic relapse within an observation period of sixty days after the cessation of treatment.

daily from the 6th to the 9th of September, inclusive; the parasites disappeared from the cutaneous blood in one day, and no relapse, either parasitic or febrile, took place during an after-treatment observation period of sixty-nine days.

TABLE VII.
Meteorological data, Accra, 1919.

| Meteorological records | Jan. | Feb. | March | April | May | June | July | August | Sept. | Oct. | Nov. | Dec. |
|---|-------|-------|-------|-------|-------|-------|-------|--------|-------|-------|-------|-------|
| Average maximum shade temperature (°F.) . | 81.74 | 84.25 | 90.83 | 87.90 | 88.22 | 87.26 | 84.58 | 84.03 | 83.90 | 85.77 | 86.63 | 86.06 |
| Average minimum shade temperature (°F) .. | 67.87 | 68.75 | 71.96 | 61.38 | 63.61 | 63.06 | 61.94 | 70.22 | 74.33 | 73.19 | 72.46 | 71.90 |
| Rainfall in inches ... | 0.09 | 1.82 | 0.54 | 4.82 | 7.52 | 1.99 | ... | ... | 0.44 | 2.42 | 0.8 | ... |

REFERENCE

STEPHENS, J. W. W., YORKE, W., MACFIE, J. W. S., BLACKLOCK, B., COOPER, C. F., and CARTER, H. F. (1918). *Ann. Trop. Med. & Parasitol.*, Vol. XII, p. 208.

IV—ORAL ADMINISTRATION OF QUININE SULPHATE TO NATIVES INFECTED WITH QUARTAN AND SIMPLE TERTIAN MALARIA

BY

J. W. S. MACFIE

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In the course of the observations on malignant tertian malaria already described, a few cases of quartan and simple tertian malaria were met with; the observations made on these cases are recorded in this paper.

As in the three previous papers, all the observations were made at Accra, in the Gold Coast, West Africa. The dates on which treatments ended are given in the tables. All the subjects were natives of West Africa infected in the Gold Coast. In every instance a diagnosis of the type of malaria was made microscopically, and in all cases trophozoites were present in the blood on the day treatment was begun. Blood examinations were made daily until the parasites disappeared from the cutaneous blood, and thereafter once a week during the after-treatment observation period. In the tables, in the Remarks columns, the days referred to are days of the after-treatment observation period.

All the subjects appeared to be healthy. The fact that they were infected with malaria was only discovered by blood examination. They lived at their own homes and went about as usual whilst under observation.

Temperature charts were not kept. The patients or their parents, however, were questioned as to attacks of fever or other illness, and in the case of school boys the attendance register was consulted.

The quinine sulphate was administered by the mouth, in solution.

QUARTAN MALARIA

Fifteen cases (Nos. 135 to 149) of quartan malaria were treated with quinine sulphate. Three of the cases received grains 20 on two, two, and nine consecutive days respectively, one received grains 10 on one day only; and the remaining eleven received grains 10 on two consecutive days. The cases ranged in age from six months to eighteen years, and included also one elderly man of sixty-five years. All the subjects excepting No. 147 were males. The results of these treatments are recorded in Table I.

TABLE I.

Summary of results of oral administration of quinine sulphate to natives in quartan malaria.

| Number of case | Age (in years) | Date of end of treatment | Daily dose of quinine sulphate in grains | Number of consecutive days on which treatment was given | Temperature fell to normal in — days after first dose | Trophozoites disappeared from cutaneous blood in — days after first dose | Trophozoite relapse occurred in — days after last dose | Febrile relapse occurred in — days after last dose | After-treatment observation period (in days) | Remarks |
|----------------|----------------|--------------------------|--|---|---|--|--|--|--|---|
| 135 | 65 | 25.7.19 | 20 | 2 | Apyrexia | 1 | . | ... | 61 | |
| 136 | 18 | 11.9.19 | 10 | 2 | Apyrexia | Same day | .. | .. | 60 | |
| 137 | 17 | 11.9.19 | 10 | 2 | Apyrexia | 1 | 57-60 | .. | 60 | |
| 138 | 14 | 17.9.19 | 10 | 2 | Apyrexia | 2 | ... | .. | 61 | |
| 139 | 13 | 17.9.19 | 10 | 2 | Apyrexia | 2 | 52-57 | ... | 61 | |
| 140 | 12 | 17.9.19 | 10 | 2 | Apyrexia | 1 | ... | ... | 61 | |
| 141 | 12 | 17.9.19 | 10 | 1 | Apyrexia | 1 | 2-9 | ... | 61 | |
| 142 | 11 | 23.9.19 | 10 | 2 | Apyrexia | 1 | . | ... | 62 | |
| 143 | 10 | 23.9.19 | 10 | 2 | Apyrexia | 1 | ... | ... | 62 | |
| 144 | 8 | 28.10.19 | 10 | 2 | Apyrexia | 2 | ... | ... | 50 | |
| 145 | 6 | 28.10.19 | 10 | 2 | Apyrexia | 2 | ... | ... | 50 | |
| 146 | 5 | 28.10.19 | 10 | 2 | Apyrexia | 2 | 23-29 | .. | 50 | |
| 147 | 2 | 8.7.19 | 20 | 9 | Apyrexia | 3 | (21) | .. | ... | Oral administration of quinine sulphate grains 10, restarted on the ninth day and given daily; relapse occurred in spite of this. |
| 148 | 1 | 1.7.19 | 20 | 2 | Apyrexia | Same day | ... | ... | 85 | Fever on the 50th day; blood negative on the 60th day. |
| 149 | 6/12 | 30.7.19 | 10 | 2 | Apyrexia | 3 | 8-14 | ... | 56 | |

In all the cases treatment was begun during an apyrexial period.

Trophozoites disappeared from the cutaneous blood either on the day treatment was begun or in one to three days. None of the cases had fever during treatment.

Relapses. Six of the fifteen cases had parasitic relapses. In two cases which did not relapse (Nos. 144 and 145) the after-treatment observation period was less than sixty days, namely fifty days. The percentage of parasitic relapses was, therefore, minimum 40, maximum 53. The case to which only a single dose of quinine sulphate grains 10 was given relapsed at once. Two of the three children under five years of age relapsed within a month.

So far as could be ascertained, none of the cases had febrile relapses. In those cases which did not relapse parasitically the length of time each was under observation after the parasitic relapse can be calculated from the data given in the table.

SIMPLE TERTIAN MALARIA

Simple tertian malaria is uncommon at Accra, and during this investigation only five cases (Nos. 150 to 154) were identified. To three of these cases quinine sulphate grains 10 was administered on two consecutive days; to two quinine sulphate grains 20 on two and three consecutive days respectively. The ages of the subjects ranged from one year to thirty-five years; they were all males. The results of these treatments are recorded in Table II.

TABLE II.

Summary of results of oral administration of quinine sulphate to natives in simple tertian malaria.

| Number of case | Age (in years) | Date of end of treatment | Daily dose of quinine sulphate in grains | Number of consecutive days on which treatment was given | Temperature fell to normal in — days after first dose | Trophozoites disappeared from cutaneous blood in — days after first dose | Trophozoite relapse occurred in — days after last dose | Febrile relapse occurred in — days after last dose | After-treatment observation period (in days) | Remarks |
|----------------|----------------|--------------------------|--|---|---|--|--|--|--|--|
| 150 | 35 | 26.7.19 | 20 | 3 | Apyrexia | 2 | ... | ... | 60 | No fever. Quinine and arsenic treatment started on the 12th day. |
| 151 | 16 | 11.9.19 | 10 | 2 | Apyrexia | 1 | 36-42 | ... | 60 | |
| 152 | 16 | 11.9.19 | 10 | 2 | Apyrexia | 1 | ... | ... | 60 | |
| 153 | 5 | 31.7.19 | 10 | 2 | Apyrexia | Present throughout | ... | ... | ... | |
| 154 | 1 | 1.7.19 | 20 | 2 | Apyrexia | Same day | ... | ... | 85 | |

In all the cases treatment was begun during an apyrexial period.

Trophozoites were present throughout in one case; in the other four they disappeared from the cutaneous blood either on the day treatment was begun or in one to two days. None of the cases had fever during treatment.

Relapses. In one case the trophozoites did not disappear from the cutaneous blood; this case was a young child. Of the remaining four cases one had a parasitic relapse, but no febrile relapse, within an after-treatment observation period of sixty days.

CONCLUSION

These observations, so far as they go, indicate that in quartan and simple tertian malaria, as in malignant tertian malaria, the percentage of relapses in natives other than very young children treated in the tropics is decidedly less than in Europeans treated in England.

ON THE RESULTS OBTAINED FROM SURVEYS FOR BREEDING-PLACES OF TREE-HOLE MOSQUITOES IN LIVERPOOL AND NEIGHBOURHOOD

BY

B. BLACKLOCK

AND

HENRY F. CARTER

(Received for publication 18 May, 1920)

PLATE I

With a view to determining the frequency with which breeding-places of *Anopheles plumbeus*, Steph., occur in trees, a series of surveys of the trees in certain areas has been made. Although the primary object of the work was to obtain such information regarding *A. plumbeus*, observations on *Ochlerotatus geniculatus*, Ol., are also recorded as the close association of these two species (*i.e.*, their coincident distribution and very similar breeding habits) renders the capture of larvae of the latter inevitable in such an investigation in this district.

METHOD OF CONDUCTING THE SURVEY

The work was performed during the months of March and April, 1920, in areas taken at random. No preference was given to localities in which breeding-places of *A. plumbeus* had previously been found; in fact, one area in which earlier work indicated exceptional abundance was excluded.

The method adopted in each survey was to examine systematically and consecutively every tree within the area chosen, provided that the diameter of the bole, at five feet from the ground, measured not less than, approximately, six inches. Except in five trees which were examined up to a height of thirty-two feet, our observations were limited to holes, or other places apparently capable of holding water, situated not more than twenty-five feet from the ground. From ground level it was often difficult to decide whether or not a definite hole existed at places where branches had been cut or broken

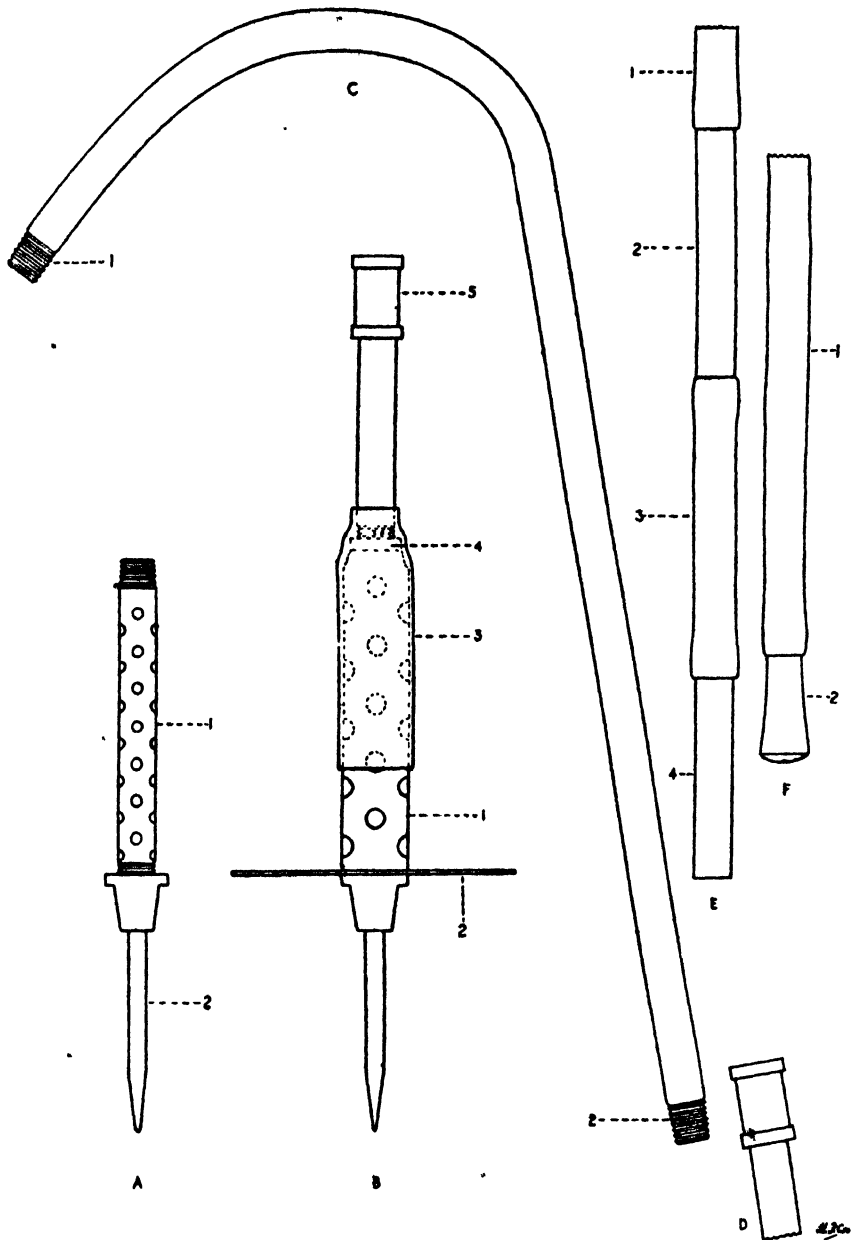
off high up on a tree. All places, therefore, where a cavity appeared to exist, and all forks and clefts where water might possibly lodge, were tested. After each examination in which a positive result was obtained by means of the apparatus described below, the instrument was thoroughly cleansed with water in order to exclude any possibility of larvae remaining in the tubes and being washed down when the next water-containing place was examined.

No correlation of breeding-places with acreage has been made, or is yet possible, owing to the irregular distribution of the areas surveyed.

APPARATUS AND METHOD OF USE

The necessity for some special apparatus for the systematic examination of possible breeding-places of *A. plumbeus* was impressed upon us by our search for larvae during the summer of 1919. Cavities situated low down (not higher than six feet) can be examined, more or less successfully and rapidly, with a spoon or ladle, provided the orifice is sufficiently large; but for holes or forks at higher levels this method of examination, involving, as it does, climbing or the use of a ladder, is too exhausting and slow to be of practical value. By means of a siphoning apparatus, such as that shown in the figure, it is possible to draw off the water contained in holes situated at any height with comparative ease and rapidity. Such an instrument has further advantages over spoons and ladles particularly when detailed work is required. The head of the siphon can be introduced into apertures too small to admit a spoon, and, by attaching lengths of metal tubing, can be extended to the bottom of a cavity without difficulty. Moreover, when much water is present, the suction produced by siphoning draws into the apparatus numbers of larvae which would ordinarily escape capture with a spoon, the use of which continually disturbs the surface layers and causes the larvae to remain, for the most part, near the bottom.

The apparatus consists of a narrow copper tube curved at the top, with the last three inches of the curved portion perforated by numerous small holes ($\frac{1}{16}$ th inch diameter). This perforated portion is separated from the rest of the tube and provided at both ends with threads which screw into suitable sockets. In



Apparatus for collecting tree-hole mosquito larvae. A, 1, inner perforated tube; 2, solid metal point. B, 1, outer perforated tube; 2, washer (3-inch diameter); 3, rubber collar; 4, socket into which the top of the inner tube screws; 5, socket for attachment to upper end (C 1) of curved tube. C, curved tube; 1, upper end; 2, lower end for attachment of extensions. D, head of first extension. E, rubber tubing (1 and 3) provided with glass windows (2 and 4). F, rubber tubing (1) to which a mouth-piece (2) or a syringe is attached. (Reduced one-half).

order to give this part of the instrument greater power of penetration into the rotten wood and débris contained in the tree-holes, a pointed piece of solid iron is affixed to its lower end. The small perforations, however, tend to become blocked with leaves and other material, and, to reduce this tendency as much as possible, the part in question is guarded by an outer tube with larger perforations ($\frac{1}{4}$ th inch diameter), and also is fitted at its lower extremity with a washer. The washer acts by pressing into the rotting material and clearing a space round the perforated tubes into which space the water may run freely. Washers of thin sheet iron, and of two and three inches diameter, were found most convenient and were used whenever the orifice was large enough to allow their introduction. The upper portion of the guard-tube is covered by a rubber collar which can be slipped up or down so as to leave only as many perforations exposed as are required. By adjusting this collar it is sometimes possible to obtain siphoning even when but little surface water is present in a hole. The required height is attained by screwing on successive four-foot lengths of brass tubing, the first being connected with the other end of the curved tube referred to above. Attached to the last extension-length is a piece of rubber tubing, about three feet long, into the upper portion of which are inserted, with an interval between, two windows of glass tubing; the lower end of the tubing is either connected with a Higginson syringe or provided with a mouth-piece.

All the above-mentioned parts of the instrument are necessarily detachable in order that adjustment, according to the character of place to be tested, may be made, and that thorough cleaning may be given when required.

When the head of the apparatus has passed into the cavity and presence of water has been determined—either by removing the head-piece from the hole or better by blowing into the tube, when bubbling will be heard—suction is exerted, and directly the fluid from the tree-hole is observed at the first window, the piece of rubber tubing immediately below it is compressed and the tube below the second window detached. The fluid is then run into a net fitted with a glass bottle.

The difficulties attending the use of this apparatus are not great

except in a high wind, when the tubes bend somewhat and render it difficult to direct. More especially is this the case when an extension to the head is used for deep holes situated high up. Under such conditions a leaden weight with a long string attached was thrown over a bough above the hole and the free end of the string tied to the head of the instrument, which was then easily pulled up to the neighbourhood of the hole. Another difficulty sometimes occurs when a hole in which little rotting has taken place contains only a small quantity of water and is so hard at the bottom that the solid point cannot penetrate; in this case the point can be removed, and with it if necessary the inner perforated tube, while the rubber collar is slipped down.

The apparatus has proved most satisfactory, and was particularly efficient in cases where a strong flow of water was obtained, the larvae then being drawn in, in large numbers, by the powerful suction. It has failed to siphon, however, on two occasions when water was present in considerable quantity; on each occasion a view of the hole was subsequently obtained by climbing or the aid of a ladder. The first failure was due to the fact that a water-logged bird's nest was present in the hole and a great quantity of glutinous material and grass were mixed with the water; no larvae were found. The second failure was partial, and was due to blockage of the perforated tubes by a very dense flocculent suspension of decayed wood; a small amount of the material, containing a few larvae of *O. geniculatus*, was obtained at the beginning of the operation, but no larvae of *A. plumbeus* were brought down although they were abundant in the hole.

ANALYSES OF RESULTS OBTAINED

In Table I are given the numbers of the different trees encountered in each of the six surveys. The first four surveys were done in the suburbs of Liverpool, within the four-mile limit, number five was made at Knowsley Park, six miles from the centre of the city, number six at Delamere, Cheshire.

As regards the first group (surveys one to four), it will be noted that oaks form an insignificant proportion of the trees; in survey six—Delamere Forest—the most striking fact is the entire absence of elms, horse-chestnuts and sycamores, trees in which, as will be

seen later (Table III) breeding-places of *A. plumbeus* and *O. geniculatus* were most commonly found. It is necessary to observe that the vast majority of the oaks and beeches dealt with in this investigation were comparatively young trees, and it is,

TABLE I.

Numbers of the different trees encountered in each survey.

| Survey | Sycamore | Oak | Beech | Elm | Birch | Spanish Chestnut | Fir | Lime | Horse Chestnut | Various | Unidentified | |
|-----------|----------|-----|-------|-----|-------|------------------|-----|------|----------------|---------|--------------|------|
| 1 | 24 | 7 | 32 | 5 | 2 | 7 | 2 | 9 | 16 | 31 | 2 | 137 |
| 2 | 63 | 3 | 123 | 38 | 4 | 7 | 1 | 22 | 16 | 25 | 1 | 303 |
| 3 | 88 | 2 | 72 | 114 | 4 | 9 | 51 | 3 | 12 | 54 | 1 | 410 |
| 4 | 32 | 3 | 56 | 28 | 1 | 2 | 17 | 4 | ... | 7 | ... | 150 |
| 5 | 293 | 378 | 159 | 67 | 44 | 39 | 11 | 61 | 15 | 100 | 2 | 1169 |
| 6 | ... | 88 | 3 | ... | 114 | 95 | 22 | ... | ... | 9 | ... | 331 |
| Total ... | 500 | 481 | 445 | 252 | 169 | 159 | 104 | 99 | 59 | 226 | 6 | 2500 |

therefore, probable that surveys conducted in areas where old oaks and beeches are common would yield materially different results. Under the heading 'various' are included many species of trees, the most numerous of which were hawthorn (41) and holly (28).

In Table II are given the results of examining all places in the trees which, from ground level, appeared capable of lodging water. Twenty-seven per cent. of all such places tested contained water; they comprised eighty-three holes containing water in seventy-four trees, and fifty-one forks or clefts containing water in forty-six trees. It must be observed, however, that in the category 'holes containing water' we have included at least twenty in which the total amount of water obtained was not more than two ounces. Remarkable variation in the number of holes found in the trees is seen, for example in survey one, nine holes containing water were found in one hundred and thirty-seven trees, whereas in survey six, no hole containing water was found in three hundred and thirty-one trees. The explanation of this variation is, we believe, to be sought less in



FIG. 1



FIG. 2

SIPHON APPARATUS IN USE

the character of the area—suburban or country—or in the tended and untended state of the trees, than in the species of tree present in the area under survey. Rot-holes in elms, horse-chestnuts, and sycamores were found to contain water more frequently than those in some other species; for example, in the limited number (about eighty) of old oaks examined rot-holes which did not contain water were numerous. Spanish chestnuts, firs, birches and young oaks presented few rot-holes.

TABLE II.
Analysis of examinations made during each survey.

| Survey | Total trees examined | Trees in which places were tested | Places tested | HOLES | | | | FORKS AND CLEFTS | | | |
|------------|----------------------|-----------------------------------|---------------|-----------------------|----------------|------------------------------|----------------|----------------------------------|----------------|---|----------------|
| | | | | Total holes examined* | Trees involved | Total holes containing water | Trees involved | Total forks and clefts examined* | Trees involved | Total forks and clefts containing water | Trees involved |
| 1 | 137 | 33 | 46 | 28 | 22 | 9 | 8 | 18 | 14 | 5 | 5 |
| 2 | 303 | 63 | 73 | 49 | 43 | 14 | 13 | 24 | 23 | 10 | 10 |
| 3 | 410 | 62 | 91 | 85 | 56 | 19 | 17 | 6 | 6 | 1 | 1 |
| 4 | 150 | 51 | 77 | 34 | 26 | 7 | 6 | 43 | 31 | 15 | 10 |
| 5 | 1169 | 143 | 191 | 134 | 103 | 34 | 30 | 57 | 53 | 20 | 20 |
| 6 | 331 | 18 | 21 | 15 | 14 | ... | ... | 6 | 4 | ... | ... |
| Totals ... | 2500 | 370 | 499 | 345 | 264 | 83 | 74 | 154 | 131 | 51 | 46 |

* The interpretation of 'holes, forks and clefts examined' as used here is given on p. 116.

The frequency with which breeding-places of tree-hole mosquitoes occurred in two thousand five hundred trees is shown in Table III. In the same table are seen the species of tree which most commonly harboured these insects. *Anopheles plumbeus* was found on sixteen occasions, always in true rot-holes, thrice alone, and thirteen times associated with *O. geniculatus*. The latter species was found on nineteen occasions, eighteen times in holes and once in a fork, five times alone and thirteen times associated with *A. plumbeus*. It will be seen, therefore, that *A. plumbeus* occurred in 0.64 per cent. of the trees examined and in 19.2 per cent. of the holes containing water. Excepting the Delamere survey, the breeding-places showed almost uniform distribution.

TABLE III.

Distribution of breeding places of *A. plumbeus* and *O. geniculatus* in holes or forks and clefts among the trees examined.

| Kind of tree | Number examined | No. of holes containing water | No. of holes in which larvae of both mosquitoes were found | No. of holes in which larvae of <i>A. plumbeus</i> only were found | No. of holes in which larvae of <i>O. geniculatus</i> only were found | No. of forks and clefts containing water | No. of forks and clefts in which larvae of both mosquitoes were found | No. of forks and clefts in which larvae of <i>A. plumbeus</i> only were found | No. of forks and clefts in which larvae of <i>O. geniculatus</i> only were found |
|------------------|-----------------|-------------------------------|--|--|---|--|---|---|--|
| Sycamore ... | 500 | 23 | 5 | ... | 1 | 10 | ... | ... | ... |
| Oak | 481 | 3 | ... | ... | ... | 2 | ... | ... | ... |
| Beech ... | 445 | 15 | ... | ... | 2 | 35 | ... | ... | 1 |
| Elm ... | 252 | 25 | 4 | 2 | ... | 1 | ... | ... | ... |
| Birch ... | 169 | 2 | ... | ... | 1 | ... | ... | ... | ... |
| Spanish Chestnut | 159 | ... | ... | ... | ... | ... | ... | ... | ... |
| Fir | 104 | ... | ... | ... | ... | ... | ... | ... | ... |
| Lime ... | 99 | 2 | ... | ... | ... | 3 | ... | ... | ... |
| Horse Chestnut | 59 | 9 | 3 | 1 | ... | ... | ... | ... | ... |
| Various ... | 226 | 3 | 1* | ... | 1† | ... | ... | ... | ... |
| Unidentified | 6 | 1 | ... | ... | ... | ... | ... | ... | ... |
| Totals ... | 2500 | 83 | 13 | 3 | 5 | 51 | ... | ... | 1 |

* Ash.

† Norway Maple.

The number of places containing water, including the number of breeding-places easily visible and attainable and those which were less easily seen and approached, is shown in Table IV. In all cases the numbers are considerably greater above six feet from the ground, the number of breeding-places of *A. plumbeus* being three times as large at the higher level.

The frequency with which holes in sycamores occur at, or below, the six feet level as compared with greater heights is noteworthy. The apertures, also, are often large and conspicuous, and, in this district unless detailed examinations are made, the erroneous impression is obtained that the sycamore is the most favourable tree for harbouring these mosquitoes. Elms, on the other hand, appear

TABLE IV.

Distribution of places containing water and breeding places in trees at heights up to six feet from the ground, and from above six feet to twenty-five feet

| Kind of tree | Up to 6 feet | | | | Above 6 feet to 25 feet* | | | |
|--------------------|--------------|------------------|---------------------------------------|--|--------------------------|------------------|---------------------------------------|--|
| | Holes | Forks and clefts | Breeding places of <i>A. plumbeus</i> | Breeding places of <i>O. geniculatus</i> | Holes | Forks and clefts | Breeding places of <i>A. plumbeus</i> | Breeding places of <i>O. geniculatus</i> |
| Sycamore | 12 | 4 | 3 | 4 | 11 | 6 | 2 | 2 |
| Beech | 6 | 4 | ... | ... | 9 | 31 | ... | 3 |
| Elm | 4 | ... | 1 | 1 | 21 | 1 | 5 | 3 |
| Horse Chestnut ... | 1 | ... | ... | ... | 8 | ... | 4 | 3 |
| Others ' | 4 | 4 | ... | 1 | 7 | 1 | 1 | 2 |
| Totals | 27 | 12 | 4 | 6 | 56 | 39 | 12 | 13 |

* Of the holes in elms two were situated at 27 ft. and 28 ft. respectively (see p. 115).

at first sight much less favourable, whereas in reality they are more so; but in them, holes are often inconspicuous and usually occur at considerable heights, so that, in ordinary circumstances, they are difficult to observe.

NUMBERS OF LARVAE AND BREEDING-PLACES

Christophers and Khazan Chand (1916), who worked with the closely related *A. culiciformis*, Cog., of Southern India, write: 'At Pudupadi the larvae were found in about 10 per cent. of the tree-holes holding water. They were found both in large holes holding several gallons and in quite small holes. . . . The largest number of larvae taken in one hole was about twenty, very frequently only one or two were found.'

During the present investigation we made counts of larvae from two holes, with the following results:—

Count 1.—92 larvae; all *A. plumbeus*.

Count 2.—346 larvae; 117 *A. plumbeus*, 229 *O. geniculatus*.

The above enumerations were made as exhaustive as possible by, first, the withdrawal of as much water as could be obtained by

siphonage, and, second, the removal of all sediment, and the rotten wood from the sides of the cavity, for examination. In the first case the cavity was small—4 in. by 5 in. by 4 in. deep—and the sediment was not in large amount, so that it was possible to place the whole contents in a large jar and to collect the larvae as they rose to the surface. In the second case the cavity was extensive (13 in. by 20 in. by 17 in. deep) and contained a large amount of sediment and much decayed wood. The water and large bulk of debris from this hole were taken to the laboratory and by a process of dilution and decantation were systematically searched for larvae. In this case owing to the great mass of material and the pressure exerted in the course of transport, with resulting mortality among the larvae, it is certain that the numbers of larvae given are considerably below the numbers actually present in the cavity.

The smallest number of *A. plumbeus* larva obtained from a breeding-place by siphonage was one. But in this connection it must be stated that in the case of the first count mentioned above, in which ninety-two larvae were enumerated from the breeding-place, two only were obtained by the process of siphonage. It is evident, therefore, that our figures for breeding-places are too low, owing to the fact, stated previously, that only twenty-five feet of the trees were examined, and also because even in that twenty-five-foot height not all breeding-places are detected, since the evacuation of larvae from small breeding-places with little surface water and much sediment cannot be assured.

SIZE OF THE LARVAE

There was considerable variation in the size of the larvae present in the breeding-places, large and small forms of both species being found. On one occasion, 30th March, numerous newly-hatched *O. geniculatus* larvae were obtained from a hole in a sycamore tree.

GENERAL DISCUSSION OF RESULTS OBTAINED

In a previous paper (1920) we ventured to express the opinion that before an area can be considered free from *A. plumbeus* a careful examination of each tree in that area must be made. We are much strengthened in our belief that this opinion is correct by our experience in the surveys just completed. The examination of

two thousand five hundred trees does not sound a formidable task, but if carried out systematically, even to the height of twenty-five feet—which may be quite insufficient—it will be found to be a distinctly arduous and exhausting procedure. The winter months appear to be the best for such investigations, because there is little foliage to obstruct the view. Towards the end of April we experienced some difficulty owing to the early leaves rendering it necessary to scrutinize extremely carefully each bough. Two excellent breeding-places of *A. plumbeus* in a horse-chestnut were nearly overlooked owing to this factor.

The apparatus which we used could with advantage be improved upon, especially in regard to the screw-on joints of the extensions. The repeated screwing and unscrewing of narrow bore tubing with the hands or with pliers is irksome. Some more rapid method of fixing the extensions would reduce the labour greatly. It should be borne in mind that the joint between the extensions must stand a pull as well as a push, because frequently the curve on the head-piece catches on twigs on being drawn down.

SUMMARY

1. In a series of six surveys, five in the Liverpool district and one in Delamere Forest, Cheshire, two thousand five hundred trees were examined up to a height of twenty-five feet for breeding-places of *Anopheles plumbeus* and *Ochlerotatus geniculatus*.

2. A total of eighty-three holes and fifty-one forks and clefts containing water were found.

3. Sixteen breeding-places of *A. plumbeus* and nineteen breeding places of *O. geniculatus* were discovered; larvae of *A. plumbeus* and *O. geniculatus* were associated on thirteen occasions. Breeding-places of *A. plumbeus* occurred in 0·64 per cent. of the trees examined, and in 19·2 per cent. of holes containing water.

4. Up to a height of six feet from the ground, thirty-nine places containing water, four breeding-places of *A. plumbeus* and six of *O. geniculatus* were found; above six feet, ninety-five places containing water, twelve breeding-places of *A. plumbeus* and thirteen of *O. geniculatus* were found.

5. Elms, horse-chestnuts and sycamores provided the great majority of the breeding-places; oaks, Spanish chestnuts and firs provided no breeding-places and very few holes containing water.

ACKNOWLEDGMENTS

Our thanks are due to The Right Honourable The Earl of Derby, K.G., G.C.V.O., for permission to examine trees at Knowsley Park; to D. Hamilton, Esq., for affording us every facility in our work there; and to Dr. Rundle, for permission to examine the trees at Fazakerley Hospital.

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NOTE.—Larvae of *A. plumbeus* have been observed from the following additional localities:—

ENGLAND

LIVERPOOL DISTRICT—

Calderstones Park. Breeding place in an elm. 8.4.1920.

IRELAND

COUNTY ARMAGH—

Newry, The Glen (border of Co. Armagh and Co. Down). Breeding place in a beech. By kind permission of Mrs. Barcroft. 23.3.1920.

COUNTY DERRY—

Moyola Park, Castledawson. Breeding place in a beech (observer, Miss M. G. Thompson, M.B.). 20.4.1920.

Between Magherafelt and Moneymore. Breeding place in a horse chestnut (observer, Miss M. G. Thompson, M.B.). 20.4.1920.

Glenburn (near Portglenone, border of Co. Derry and Co. Antrim). Breeding place in a beech (observer, Miss M. G. Thompson, M.B.). 27.4.1920.

COUNTY LOUTH—

Ravensdale Park, Ravensdale. Breeding place in a beech. 20.3.1920.

Anaverna, Ravensdale. Breeding places in a beech and a fallen elm. 20.3.1920.

CROSSOCEPHALUS ZEBRAE, N.SP.

BY

WARRINGTON YORKE

AND

T. SOUTHWELL

(Received for publication 20 May, 1920)

This nematode was present in considerable numbers in the collections made from the intestine of six zebrae (*Equus burchelli*) shot by one of us in Northern Rhodesia, 1912.

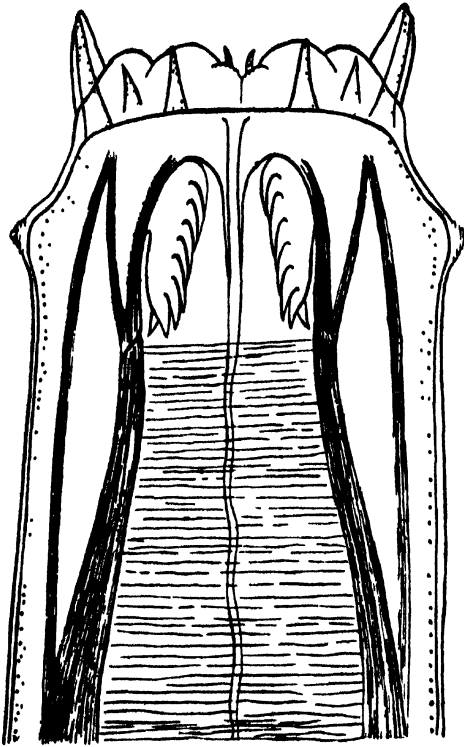
SIZE AND SHAPE. It is a moderately small species, the female being slightly larger than the male. Four sexually mature males and ten females were measured. The males were from 7·6 mm. to 8·3 mm. in length, average 8·0 mm.; the females from 7·4 mm. to 9·4 mm., average 8·6 mm.; the greatest breadth averaged, males 440 μ , females 498 μ . The anterior extremity is truncated; the posterior extremity of the male is inrolled ventrally and that of the female straight and tapering.

THE HEAD. The anterior end of the body tapers very slightly to the head, which is sharply truncated; there is no definite neck (figs. 1 and 2).

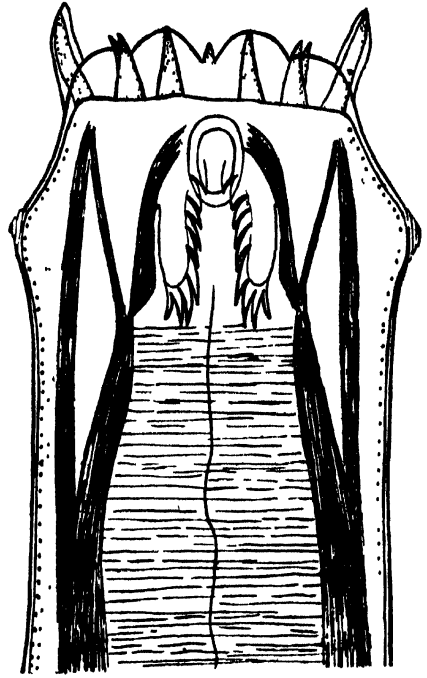
Lips. The mouth is surrounded by three lips (one dorsal and two sub-ventral), each lip, on its oral aspect, being sub-divided into three portions, and having a finely serrated edge (fig. 5).

Head papillae. There are numerous (fifteen) head papillae having a complicated arrangement as shown in figs. 1-5. The lateral papillae (*a*) are very prominent; there are four sub-median papillae (*b*) just projecting beyond the surface of the lips; there is also an additional papilla (*c*) situated at the middle of each lip; and finally there are three pairs of small papillae (*d*) arranged so that one papilla lies on each side of the junctions of the three lips.

There is no chitinated mouth capsule, but there are three pairs of pectinated laminae situated in the anterior end of the oesophagus (figs. 1 and 2). Each of these laminae bears from eight to eleven pointed teeth, the points being directed backwards when the mouth



A. M. B. del.

FIG. 1. *Crossocephalus zebræ*.Anterior extremity, ventral view, mouth closed. $\times 360$.

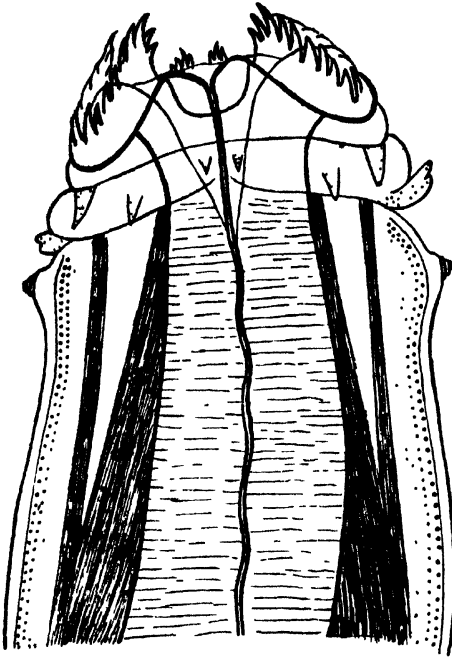
A. M. B. del.

FIG. 2. *Crossocephalus zebræ*.Anterior extremity, dorsal view, mouth closed. $\times 360$.

is closed. The mouth is, however, capable of being everted, in which condition the lips are folded back over the anterior end of the worm, the papillae being directed backwards; the anterior portion of the oesophagus then forms the anterior end of the worm, the six pectinated laminae being erected and their teeth directed forwards (figs. 3 and 4). Eversion is probably to a considerable extent brought about by the action of certain prominent longitudinal muscles which are shown in figs. 1 and 2.

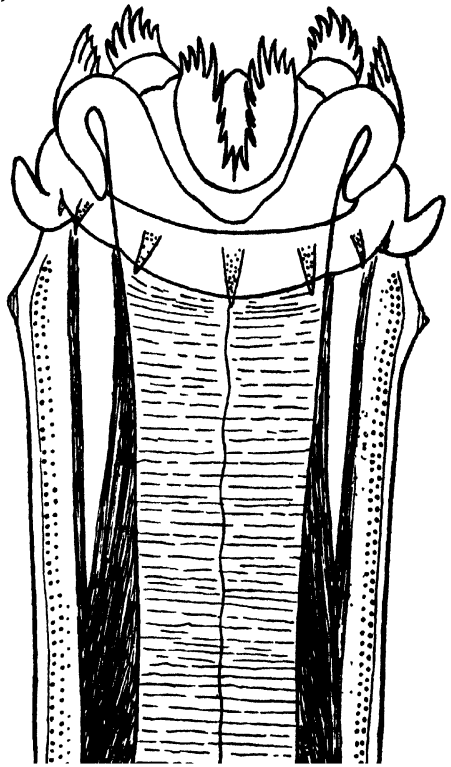
The duct of the *dorsal oesophageal* gland opens into the mouth (fig. 5. g).

OESOPHAGUS. The length of the oesophagus in seven female worms, in which the mouth was closed, varied from $1,016\mu$ to $1,085\mu$, the average being $1,045\mu$. When the mouth is open there is an apparent shortening of the oesophagus to the extent of about



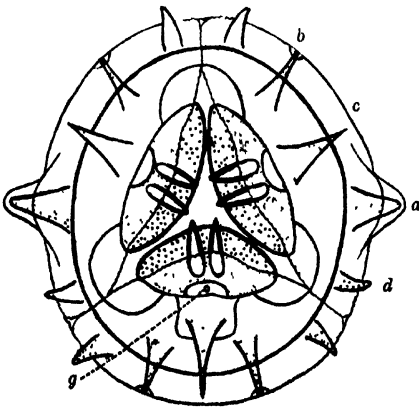
A. M. B. del.

FIG. 3. *Crossocephalus zebræ*.
Anterior extremity, ventral view, mouth everted. $\times 360$.



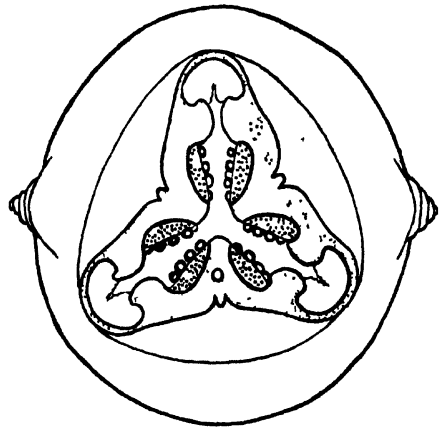
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FIG. 4. *Crossocephalus zebræ*.
Anterior extremity, dorsal view, mouth everted. $\times 360$.



A. M. B. del.

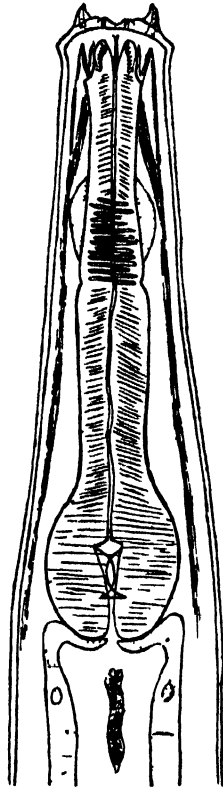
FIG. 5. *Crossocephalus zebræ*.
End-on view of head, mouth slightly everted, shewing position of the various papillae; *a* = lateral papillae, *b* = sub-median papillae, *c* = papillae on middle of each lip, *d* = paired papillae, *g* = duct of dorsal oesophageal gland. $\times 360$.



A. M. B. del.

FIG. 6. *Crossocephalus zebræ*.
Transverse section through oesophagus at level of cervical papillae. $\times 360$.

100 μ , showing the extent to which eversion of the anterior end of oesophagus proceeds. In three males, the oesophagus varied in length from 1,002 μ to 1,020 μ , average 1,013 μ ; the apparent shortening of the oesophagus in worms with the mouth open was here also about 100 μ . The ratio of the length of the oesophagus to that of the worm is about 1 to 8.



A. M. B. del.

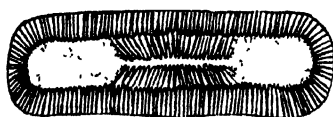
FIG. 7. *Crossocephalus zebræ*.

Anterior end showing oesophagus, ventral view. $\times 360$.

A series of yellow club-shaped bodies, possibly of a glandular nature, occur in the wall of the oesophagus; these commence about 220 μ from the anterior extremity of the worm, and they extend backwards for a distance of about 70 μ . The bulb of the oesophagus contains a valvular arrangement consisting of three ridges projecting into the lumen. The appearance of the above structures is shown in fig. 7.

EXCRETORY PORE. This is situated about twice the length of the oesophagus from the anterior extremity. In ten females the distance of the excretory pore from the anterior end of the worm varied from $1,866\mu$ to $2,110\mu$; average $2,041\mu$; and in four males from $2,027\mu$ to $2,172\mu$; average $2,110\mu$.

The pore presents the appearance of a transverse slit, and is surrounded by a pallisade-like structure consisting of cuticular ridges (fig. 8).



A. M. B. del.

FIG. 8. *Crossocephalus zebræ*.

Excretory pore. $\times 240$.

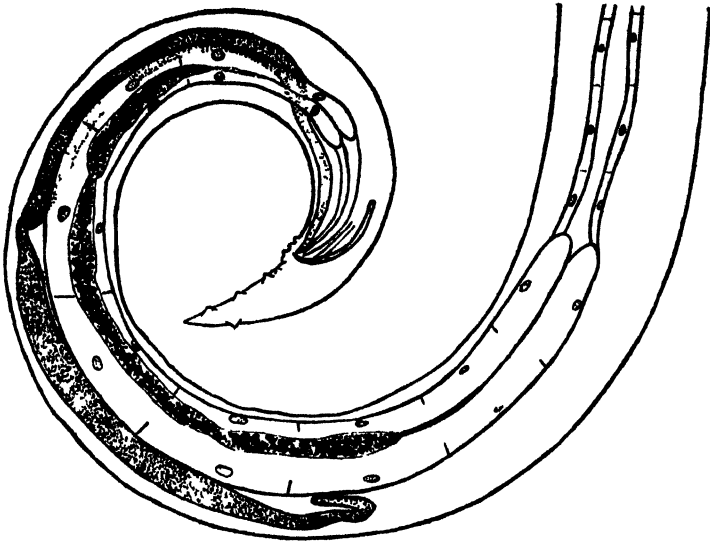
CERVICAL PAPILLAE. These are small, nipple-like projections lying very close to the anterior extremity of the worm (figs. 1 to 4).

POSTERIOR EXTREMITY OF MALE. The posterior extremity of the male is markedly inrolled, ventrally. The tail tapers to a point and is furnished with three pre-anal and five post-anal papillae, as is shown in fig. 10. There are no membranous expansions.

Spicules. The spicules are unequal in size, the larger being almost exactly twice the length of the smaller. In eight worms the length of the larger varied from 295μ to 353μ ; average 330μ , and the smaller from 145μ to 176μ ; average 165μ . The larger spicule exhibits fine transverse striations; the smaller is not striated (fig. 11). The testes and ejaculatory duct are limited to the posterior third of the worm, and are shown in fig. 9.

POSTERIOR EXTREMITY OF FEMALE. The end of the female is straight, the tail tapering to a point. In nine worms the distance from the anus to the vulva varied from 170μ to 255μ , average 195μ ; and the distance of the anus from the tip of the tail from 488μ to 617μ , average 551μ (fig. 12).

There is a single tubular ovary the anterior extremity of which lies about 300μ behind the excretory pore. The oviduct runs forward for a short distance and then dilates into a receptaculum seminis. The uterus is a thin-walled sac and reaches nearly as far

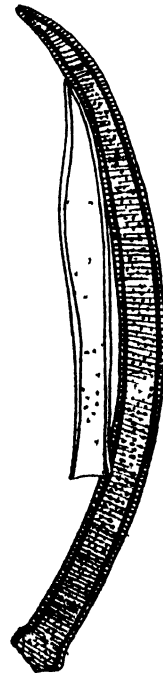


A. M. B. del.

FIG. 9. *Crossocephalus zebrae*.
Posterior extremity of male. $\times 45$.



A. M. B. del.



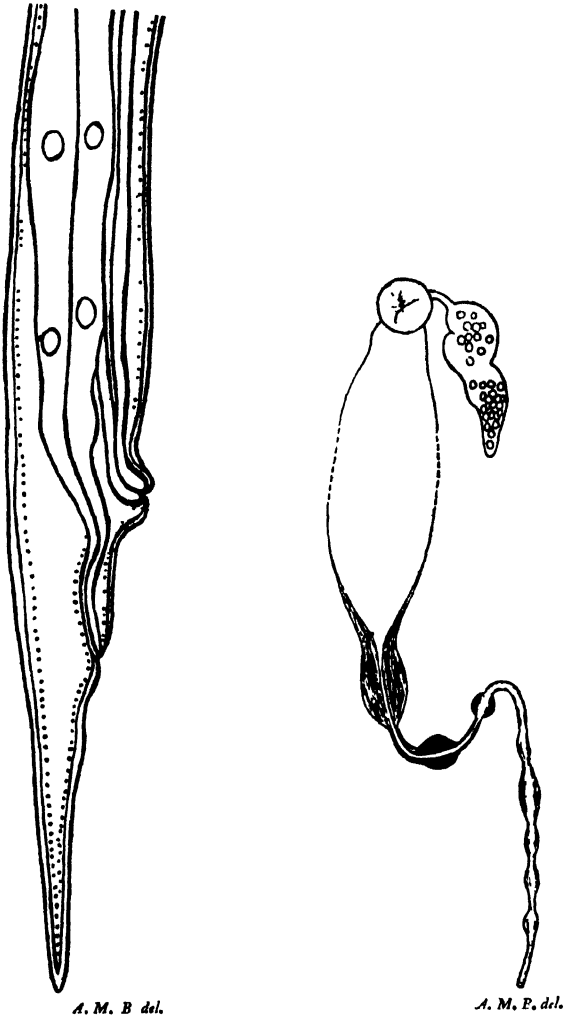
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FIGS. 10 and 11. *Crossocephalus zebrae*.

Fig. 10. Posterior end of male. $\times 120$.

Fig. 11. Spicules. $\times 360$.

posteriorly as the vulva; in gravid worms it contains one or more fully formed larvae which attain to a length equal to about half that of the parent worm; the larvae exhibit clearly the six pectinated laminae. In the posterior part of the uterus developing larvae can be seen. The vagina commences as a thick muscular tube and exhibits a beaded appearance due to numerous dilatations, it runs first posteriorly, and then anteriorly for a short course, and then directly posterior to the vulva (fig. 13).



A. M. B. del.

A. M. F. del.

FIGS. 12 and 13. *Crossocephalus zebræ*.

Fig. 12. Posterior extremity of female. $\times 120$. Fig. 13. Diagram of female genitalia.

DIAGNOSIS

This worm is probably closely related to the worm described by Von Linstow (1899) under the name *Pterocephalus viviparus*, and which was subsequently re-named *Crossocephalus* by Railliet in 1909, as the genus *Pterocephalus* was already occupied. We infer this from an examination of Von Linstow's figures rather than from a study of his written description of the worm. The six pectinated laminae of our worm obviously correspond to the '6 aufrichtbare Flügel' described and figured by Von Linstow. Other points in which the worms agree are the possession of two unequal spicules, the character of the tail in the male, and the fact that both are viviparous. These points appear to us to be sufficient to establish the identity of the genus. As regards the minute anatomy of the structure of the head, Von Linstow's description, and some of his figures, appear to us so extraordinary that we can hardly believe that they are correct; indeed, this is the view held by Gedoelst (1906), who found a number of females of what he believed to be *Crossocephalus viviparus* in the intestine of a zebra from Katanga. Apart from the minute anatomy of the head and from certain minor characters, such as the number of papillae on the tail of the male, Von Linstow's worm presents one most striking difference from ours, viz., the position of the vulva. This structure, according to Von Linstow, is situated a short distance behind the middle of the body, whilst in our specimens it was invariably placed immediately in front of the anus; this was also the case in Gedoelst's worm. The description and figures of the head given by Gedoelst differ markedly from ours in certain respects, notably in the arrangement of the papillae. Moreover, Gedoelst's description is of necessity incomplete, as the material at his disposal was limited to females, and to specimens in which the mouth was closed.

We have, then, to deal with three worms all presenting certain morphological similarities, all coming from the same host, and yet all differing in detail. The head of the worm is extremely complicated and possibly the differences may be due to faulty interpretations, and the worms may really all be the same species. But this hypothesis can hardly explain the position assigned to the vulva by Von Linstow. Unfortunately we have been unable to

examine Von Linstow's co-types, and so cannot offer a definite opinion on the matter. It appears to us, therefore, that the proper procedure is to act on the assumption that the description given by Von Linstow is correct, and to regard our worm as a new species, *Crossocephalus zebrae*.

Attention might be drawn to the fact that Baylis (1919) has recently given the name *Crossocephalus longicaudatus* to a closely allied species found in a rhinoceros from the Malay Peninsula.

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TARTAR EMETIC IN GUINEA-WORM INFECTIONS

BY

J. W. S. MACFIE

(Received for publication 22 June, 1920)

PLATE II

On a previous occasion (1920) an account was published of the effects of intravenous injections of tartar emetic in ten cases of guinea-worm infection. Since this preliminary note was written a few additional observations have been made on the same cases, and a few new cases have been treated, so that details are now available of the effects observed in twenty-three patients and on thirty-nine worms. In the present paper the results of the treatments are briefly summarised. The data regarding the cases are given in the Table. All the patients were treated at Accra. I have again to thank Dr. A. J. R. O'Brien for very kindly permitting me to make use of notes made on cases in his charge.

TECHNIQUE OF TREATMENT

Excepting on rare occasions, the tartar emetic was administered in doses of one grain each, and was given every other day. The injections were made into a vein at the bend of the elbow with a 'Record' syringe and a fine needle. The strength of the solution employed, which was made up with normal saline solution and contained 0.5 per cent. phenol, was 0.5 of a grain of tartar emetic in each cubic centimetre; this solution was found to be the most convenient and suitable for general use. The solution should always be freshly prepared before use. On one or two occasions (Cases Nos. 21, 22, 23) a stock solution which had been prepared two or three months previously was used, and was found to give disappointing results. Better results followed immediately in these cases when a freshly prepared solution was substituted. It is

possible that some of the other less successful results in earlier cases may be accounted for in the same manner.

Many of the cases were treated as out-patients. Cases treated in hospital, where they were able to rest completely, appeared to do better, however, and were sooner fit to return to their employments. This was most noticeable in patients in whom the worm had already set up a considerable degree of inflammation.

No serious ill-results were observed to follow the injections. In two cases, after four and three grains respectively, a slight general papular rash developed, which, however, disappeared when the treatment was discontinued; and Dr. O'Brien reported that one of his patients showed symptoms of collapse after injection on one occasion.

EFFECTS ON THE GUINEA WORMS

Intravenous injections of tartar emetic will kill guinea-worms and the embryos in them. In one or two cases the effect has been observed on worms situated superficially and visible beneath the skin (*e.g.*, Case No. 15; Plate II, figs. 1 and 2). In such cases the worm soon became stationary, and was slowly absorbed. The worms have also been examined microscopically at various stages of treatment by extracting small portions of them and searching them for embryos. Pieces of sixteen worms were removed and examined during the courses of antimony treatment, fourteen once only and two on two occasions. Living embryos were found in worms after two and a half, three, three, four, five, and six grains, after a previous course of six grains and one recent dose of one grain, and after ten, ten, and fourteen grains of an old preparation which was probably not of full strength. Dead or disintegrated embryos only were found in worms after four, four, four, four and a half, five, seven, and seven grains. The two worms which were examined in this manner twice showed living embryos on the first occasion, after two and a half and four grains, and only dead embryos on the second occasion, after four and a half and seven grains respectively. It is clear, therefore, that the same dose of tartar emetic does not always have the same lethal effect,

some worms being killed, and their embryos with them, by four grains, others successfully withstanding larger doses.

From these results there can, I think, be no doubt that injections of tartar emetic will kill guinea-worms and their embryos: the results are, however, not always similar. Most frequently the worms remain in the body, and are gradually absorbed. In some cases the greater part of the worm remains in the body and is absorbed, but one or more pieces of dead worm slough before the wound becomes soundly healed. In other cases the worm appears in the wound, and can be extracted without difficulty or danger. So complete was my confidence in the efficacy of tartar emetic that during treatment I frequently pulled out and broke off protruding portions of the worms in order that the remainders might retract and permit of the healing of the external wounds. No ill-effects followed this practice, which under other circumstances would inevitably have resulted in acute inflammation and abscess formation.

In several cases the treatment appeared to have the effect of bringing to the surface other guinea-worms that happened to be in the body. The worms sometimes reached the skin surface alive (Cases Nos. 15, 27), sometimes dead (Case No. 14), and sometimes they failed to make their way through to the surface, and only succeeded in getting so far that they became palpable or produced local swellings.

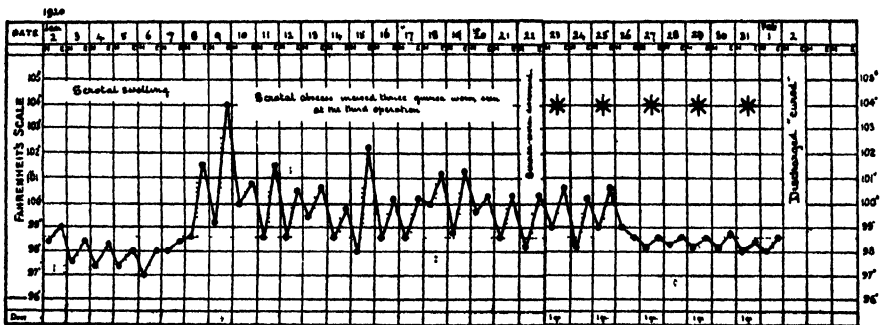
EFFECTS IN REDUCING INFLAMMATION

The injections of tartar emetic appeared to relieve inflammation as well as to kill the guinea-worms. So notable was this effect that it was thought that the treatment might prove of value in dealing with inflammation and suppuration due to other causes. No opportunity having yet occurred of putting this to the test, it may be of interest to recount here briefly two of the cases in which this action appeared to be well marked.

Case No. 13 (see Chart). This patient was admitted to hospital suffering from orchitis, and developed an abscess on the left side of his scrotum. The abscess was opened three times, nevertheless the inflammation and the fever persisted. At the third

operation a guinea-worm was seen in the scrotum, and in consequence intravenous injections of tartar emetic were started next day. The fever subsided, the inflammation was arrested, and the wounds healed after the second injection.

Case No. 14. This patient had a guinea-worm in his right foot, which was cured after the injection of four grains of tartar emetic. A fortnight later a swelling appeared in the left groin and came to a head. It was punctured, about a drachm of pus was let out, and a guinea-worm was seen. The guinea-worm was dead; no embryos were found in the discharge from the wound, and only disintegrated ones in a small piece of the worm removed for microscopical examination. As the worm and its embryos were dead, it was anticipated that the swelling would subside and that the



Temperature Chart of Case No. 13 * = intravenous injection of one grain of tartar emetic.

wound would heal without special treatment other than that usually applicable to such an abscess. This did not occur immediately, however, and four days later intravenous injections of tartar emetic were restarted. The inflammation began to subside at once, and the wound healed rapidly.

Many other cases might be instanced in which the inflammation of the affected limb and the discharge from the guinea-worm sore appeared to respond immediately to intravenous injections of tartar emetic, but in some of them there was the possibility, not present in the two cases mentioned above, that the relief of the inflammation might have been due to the killing of living embryos which had invaded the tissues.

EFFECT ON THE DURATION OF THE INFECTION

It is not possible to estimate accurately the reduction in the duration of the illness due to guinea-worm infections effected by the use of intravenous injections of tartar emetic because there are not available data showing the usual duration, and because, naturally enough, the length of the illness depends very largely on the condition of the patient on admission, the nature of the treatment previously adopted, the site of the worm, and on many other circumstances. Cases similar to those dealt with in this paper when treated by the older methods are generally reckoned, however, to be unfit for work for about six weeks, very much longer if a succession of worms develops.

In those of our cases in which the infection was single and was not complicated by some other affection, the average length of time from admission to discharge was about twelve days. In some cases it was less, in some considerably more.

It may be affirmed, therefore, that the treatment effects a real reduction in the duration of the illness, besides reducing the liability to the more serious consequences attending other forms of treatment.

THE DOSE OF TARTAR EMETIC REQUIRED

Definite proof has, I believe, been obtained that the intravenous injection of four grains of tartar emetic is sufficient in many cases to kill a guinea-worm and the embryos in it. This dose, however, is not invariably sufficient. It has been noted also that in the same patient, after one guinea-worm has been cured by this treatment, other guinea-worms may make their appearance at the surface of the body. These rather conflicting results suggest that all guinea-worms are not equally susceptible to the drug, and that either in certain stages of their development, or in certain situations in the body, they are less easily killed than when they have come naturally to the surface for the purpose of discharging their embryos.

It may be suggested that to give every other day an injection of one grain of tartar emetic until a total dose of six grains has been administered would be found to be satisfactory treatment in most cases. In the event of relapse or recurrence, a second course should

be given. Further experience may show that, in some cases at any rate, longer courses are necessary, and that it is preferable to augment both the individual and the total dose, but the cases collected in the Table show that very satisfactory results may follow courses of as few as four or five injections of one grain each.

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TABLE

R = right; L = left; C = cured (in the clinical sense).

An injection, unless otherwise stated, is 1 grain of tartar emetic.

| Case No. | Approximate age in years | Total dose of tartar emetic given; in grains | Patient last seen days after cessation of treatment | Site of the guinea-worm | Condition when treatment started | Result | Remarks |
|----------|--------------------------|--|---|-------------------------|---|----------------|---|
| 1 | 20 | 9 | 48 | L. leg | Worm broken; leg swollen and painful; fever | C. | No bits of worm came away. |
| 2 | 23 | 5 | 30 | L. leg | Whole worm in the body | C. | Worm did not come away. |
| 3 | 30 | 6 | 35 | L. foot | Whole worm in the body | Worm extracted | Sore closed, but re-opened a week after cessation of treatment, worm protruded and was wound out. |
| 4 | 24 | 4 | 44 | R. leg | Whole worm in the body; leg much swollen | C. | Worm did not come away |
| 5 | 15 | 5½ | 21 | R. leg | Whole worm in the body | C. | A bit of the worm was pulled out and broken off, the remainder did not come away |
| 6 | 47 | 6 | 68 | L. leg | Worm broken; sore granulating and discharging | C. | No bits of worm came away |
| 7 | 30 | 6 | 70 | L. foot | Worm broken, foot swollen | C. | A bit of worm sloughed out three days after last dose; sore then healed. No relapse |
| | | | | L. foot | Whole worm in the body | Worm extracted | Worm appeared after the third injection; extracted |
| 8 | 18 | 4 + 7 | ... | L. foot | Whole worm in the body | C. | After four injections patient went away for a month; returned with sore open and septic, and leg swollen. Rapidly healed after treatment resumed. Returned with a guinea-worm in the right foot also. |
| | | | | R. foot | Whole worm in the body | Worm extracted | After 4 grains, piece of worm pulled off: embryos in it were dead. After 7 grains the rest of the worm was easily extracted |
| 9 | 30 | 3½ | 69 | R. foot | Worm broken; foot swollen and tender | C. | No bits of worm came away. |
| 10 | 22 | 4 | ... | R. foot | Worm broken; foot swollen and tender | Improved | Patient went away and was not seen again. |
| 11 | 20 | 6 | 52 | R. foot | Worm broken | Worm extracted | |
| 12 | 26 | 5 | 26 | R. foot | Worm broken; foot swollen and inflamed | C. | No bits of worm came away |
| 13 | 18 | 5 | 10 | Scrotum | Worm broken; scrotal abscess. Fever | C. | Temperature fell to normal and abscess wound healed after the second injection |

TABLE—continued

| Case No. | Approximate age in years | Total dose of tartar emetic given; in grains | Patient last seen days after cessation of treatment | Site of the guinea-worm | Condition when treatment started | Result | Remarks |
|----------|--------------------------|---|---|-------------------------|--|----------------|---|
| 14 | 15 | 4 + 4 | 42 | R. foot | Worm broken; foot and leg swollen | C. | No bits of worm came away. A fortnight after discharge from hospital a swelling appeared in the left groin forming an abscess. |
| | | | | L. groin | Whole worm in the body | C. | Groin swelling opened and a piece of guinea-worm removed before resuming treatment; it contained only dead embryos. Abscess healed rapidly, and swelling subsided. |
| 15 | 18 | 6 + 5½ | 44 | R. side of body | Whole worm in the body | C. | Worm subcutaneous: slowly absorbed, neither visible nor palpable when last examined. Ten days after the first course of treatment the second worm appeared. |
| | | | | R. groin | Whole worm in the body | C. | Piece of worm removed after the first injection: embryos in it living. Healed after the second injection. A week later two inches of dead worm sloughed, then sore healed again and has not given further trouble |
| 16 | 48 | 5 | 6 | L. foot | Whole worm in the body | C. | Six inches of disintegrated worm extracted after 4 grains had been given; the remainder did not come away. A second worm appeared a week after discharge. |
| 17 | 22 | 3 + 3 | ... | R. foot | Whole worm in the body; abscess | Improved | A week intervened between the third and the fourth injections; living embryos were still present when treatment resumed. Practically healed when last dose given, after which patient ceased to attend for treatment. |
| 18 | 20 | 3 | ... | R. foot | Whole worm in the body; foot swollen | C. | Cleared up after two injections |
| 19 | 20 | 3 | 27 | L. leg | Whole worm in the body; leg swollen, abscess | Worm extracted | Six inches of worm pulled off after first injection. Four days after last injection abscess burst and rest of worm was easily extracted. No further trouble. |
| 20 | 34 | 6 | 10 | L. leg | Whole worm in the body; leg greatly swollen | C. | Inflammation and swelling subsided rapidly. Worm did not come away. |
| 21 | 20 | 4 + 5 (old solution used for first six injections) | ... | R. leg | Whole worm in the body | C. | Healed rather slowly after four injections |
| | | | | R. ankle | Whole worm in the body | C. | Healed partially after the first course. A month later relapsed; living embryos present. Healed rapidly after treatment resumed |
| | | | | R. foot | Whole worm in the body | Improved | Healed partially after the first course. Relapsed a month later. A piece of worm examined after the grains of second course contained only disintegrated embryos. Still under observation; doing well |

TABLE—continued

| Case No. | Approximate age in years | Total dose of tartar emetic given; in grains | Patient last seen days after cessation of treatment | Site of the guinea-worm | Condition when treatment started | Result | Remarks |
|----------|--------------------------|--|---|-------------------------|--|----------------|---|
| 22 | 23 | 9 (old solution used for first six injections) | 5 | R. foot | Whole worm in the body | C. | Healed after the eighth injection |
| | | | | R. leg | Whole worm in the body; abscess | Improved | Four inches of dead worm sloughed two days after the cessation of treatment. Still under observation: doing well, almost healed |
| | | | | Perineum | Whole worm in the body; abscess | Worm extracted | Abscess burst two days after cessation of treatment and complete dead worm came away. Still under observation: doing well, almost healed |
| | | | | L. foot | Whole worm in the body; abscess | Improved | Worm did not come away. Almost healed |
| 23 | 24 | 6 + 4 + 6 (old solution used for first fourteen injections) | 5 | R. leg | Whole worm in the body; leg greatly swollen; fever | C. | Treated as out-patient during first course, then admitted to hospital and given four more injections which resulted in rapid healing. Worm did not come away. A week later patient returned with seven guinea-worms, and third course was started |
| | | | | Abdomen | Whole worm in the body | Improved | Worm subcutaneous, small opening at head. Sore nearly healed; still under observation |
| | | | | Abdomen | Whole worm in the body | Improved | Worm subcutaneous. Still under observation: worm palpable and still viable at one spot |
| | | | | Back | Whole worm in the body | C. | Worm subcutaneous. Apparently cured; only just palpable, and apparently being absorbed |
| | | | | L. thigh | Whole worm in the body | Improved | Worm subcutaneous. Still under observation; worm palpable but apparently being absorbed |
| | | | | L. leg | Whole worm in the body | C. | Healed rapidly |
| | | | | L. foot | Whole worm in the body | Improved | Still under observation: nearly healed |
| | | | | R. foot | Whole worm in the body | Improved | Still under observation: nearly healed |

EXPLANATION OF PLATE II

- Fig. 1. Case No. 15, showing the guinea-worm, situated under the skin of the chest and abdomen, as it appeared at the time treatment was started.
- Fig. 2. The same case after administration of four grains of tartar emetic. The upper (tail) portion of the guinea-worm was at this time invisible and almost impalpable.



FIG. 2



FIG. 1

THREE CASES OF CARDIAC ANEURYSM IN NATIVE BOYS OF THE GOLD COAST

BY

J. W. S. MACFIE

AND

A. INGRAM.

(Received for publication 19 August, 1920)

PLATE III

In this note we record in the briefest manner three cases of aneurysm of the heart in native boys of the Gold Coast, West Africa, which have come under our notice during the last four years. We have to thank Dr. H. T. Palmer, Dr. C. H. D. Ralph, and Dr. G. E. H. Le Fanu for the opportunity of examining the first, the second, and the third, respectively, and for furnishing the notes made at the autopsies, and we take this occasion of expressing our indebtedness to them.

CASE I. A native boy, aged seven years, who died suddenly in the early hours of the morning when getting from his bed. The boy was not seen before death, and no history of previous ill-health was reported by the parents.

At the autopsy the body was found to be that of a well developed boy. No macroscopical lesions were observed excepting those of the heart. The pericardium was full of blood, which had issued from a rupture, about a quarter of an inch long, in an aneurysm near the apex of the left ventricle. There were no pericardial adhesions. The cavity of the aneurysm contained well-formed fibrinous clot.

The *heart* (Plate III, fig. 2) was not hypertrophied; there was no valvular disease. At the apex of the left ventricle was a small saccular aneurysm extending on the surface from the tip of the heart downwards for about 15 mm., then outwards, and upwards on the left side for about 25 mm., and internally communicating with the

chamber of the left ventricle by a narrow opening. The aneurysm was composed of two chambers incompletely separated, a small cavity filled with fibrous clot at the very apex of the heart, and a larger cavity a little to the left side. In the photograph the larger cavity is shown after it had been laid open by a longitudinal incision. The larger cavity measured 25 mm. by 15 mm. by 15 mm., its wall was very thin, especially at its lower and anterior pole, where it had given way. The inner surface was partly covered by fibrinous clot.

Sections of the wall of the left ventricle showed a considerable degree of interstitial myocarditis. The aorta appeared to be healthy. The left coronary artery showed a slight degree of endarteritis together with some degeneration of the inner coat.

CASE II. A native boy, aged six years, who suddenly fell dead outside school. He had not been pushed or struck, and, it was stated, had never been ill nor attended by a doctor.

At the autopsy the body was found to be well nourished, without signs of injury or violence. In addition to the condition of the heart, presently to be described, there were found a few adhesions at the apex of the right lung, an enlarged spleen, and a few round worms (*Ascaris lumbricoides*) in the intestine; the organs of the body appeared to be otherwise healthy. The pericardium was full of blood clot. When this was cleared away it was seen that the blood had escaped from the heart through a small opening near the apex of the left ventricle. There were no pericardial adhesions. The posterior mediastinal glands were enlarged, and several of them contained yellow caseous matter.

The *heart* (Plate III, fig. 1) was not hypertrophied. On the left side, a little posterior to the middle of the ventricle and spreading on to the front of the heart, was a swelling nearly 30 mm. in diameter and raised about 10 mm. above the normal level of the heart, on this swelling were three distinct nodules, one anterior and two posterior. Connected with the swelling, at its lower and external margin, was a sac-like prolongation, about 17 mm. long and 14 mm. broad at its base, which projected freely on the posterior aspect of the heart; at its free margin, where the wall was very thin, was a small tear. On incising the heart it was found that the external bulgings were caused by two aneurysms, both opening a little above and to the

outer side of the apex of the left ventricle at the roots of the papillary muscles, the one anterior and the other posterior, but quite distinct. The latter had ruptured, thus bringing about the death of the boy.

At the base of the heart, lying between the ventricles just below the aortic orifice, was a rounded mass about 10 mm. in diameter which had the appearance of a gumma, both to the naked eye and in sections. The myocardium in the neighbourhood of the gumma showed a considerable increase of fibrous tissue. Sections of the wall of the left ventricle showed a slight degree of interstitial myocarditis. The left coronary artery showed a slight degree of endarteritis and degeneration of the inner coat.

CASE III. A native boy, aged twelve years, who died suddenly during a quarrel with another boy. At the autopsy it was noted that, in addition to the presence of an aneurysm of the heart, the liver and lungs showed signs of chronic inflammatory changes, and that the arteries were sclerosed. Death was due to heart failure.

The *heart* (Plate III, fig. 3) appeared to be slightly hypertrophied. With the exception of the apical portion, practically the whole of the posterior wall of the left ventricle was occupied by a convex dilatation of almost stony hardness; this dilatation formed a dome-shaped structure, with a diameter of about 40 mm. and a height of about 30 mm. In the photograph it is shown as seen from the inner aspect after the heart had been incised. The outer surface was smooth; there were no pericardial adhesions. The inner surface was irregularly studded with calcareous nodules, some of which were smooth, others very rough; it was divided by an oblique ridge of no great prominence into an upper and a lower portion. The middle part of the dome was thin and more or less translucent, and appeared to be bony.

On the aorta were numerous small patches of atheroma, which in sections showed calcification and proliferation of the inner coat. Sections of the wall of the left ventricle external to the aneurysm showed practically no fibrosis. The left coronary artery showed a slight degree of endarteritis, slighter than in either of the two preceding cases.

It is somewhat remarkable that these three cases, which are the only cases of cardiac aneurysm that we have hitherto met with in

the Gold Coast, were young boys (six to twelve years), for Legg (1883) considered that aneurysm of the heart was a 'disease of middle and advanced life, rather than a disease specially common below thirty, as Thurnam believed,' and Hall (1903) records only a solitary case under twenty, and this one due to trauma. At the time Legg wrote, the youngest case known was 'a little girl of twelve (who) died suddenly while at play from the bursting of the aneurysm.'

According to Hall, the aneurysms are nearly always single. It is interesting to note, therefore, that in these three cases, one aneurysm was bilocular, and one double. In all the three cases the aneurysms formed definite swellings or tumours on the surface of the heart, but there were no pericardial adhesions. In two of the cases the myocardium showed some degree of fibrosis, in the third no such change was apparent in the sections cut.

Death was sudden in all three cases, and, so far as could be ascertained, was not preceded by any symptoms of illness. Two of the boys died from the rupture of the aneurysm into the pericardium. In this connexion, it may be recalled that on a previous occasion (1917) attention was drawn to the fact that in the Gold Coast perforation into the pericardium of small aneurysms of the intra-pericardial aorta is not a very uncommon cause of sudden death in adult natives previously thought to be in good health and certainly capable of hard physical work.

Hall brings forward coronary endarteritis as 'the great cause of aneurysms of the left ventricle, and, with hardly any exceptions, as the sole cause of aneurysms at the apex.' The result, he says, is 'either sudden obstruction leading, if the collateral circulation is insufficient, to infarction, or gradual obstruction leading to replacement of the myocardium by fibrous tissue, which may or may not yield later to the blood pressure, and cause aneurysm,' and the most usual sequence of events 'a gradual stenosis of the artery by disease, to which is superadded thrombosis, causing sudden complete obliteration.' Particular interest, therefore, attaches to the condition of the coronary arteries in these cases. In all three the condition was similar; there was a slight degree of endarteritis together with some degeneration of the inner coat. Professor Ernest Glynn has been so kind as to examine the sections of the

arteries, and is of the opinion that the condition was not characteristic of syphilitic endarteritis, but that in view of the gumma-like tissue detected in the second case it was possibly of this nature.

Apart from the conditions of the heart, no other, or only slight macroscopic lesions were observed at the autopsies on these boys, who appeared to have been otherwise healthy, and, so far as could be ascertained, had not previously suffered from serious illness. It may be confidently assumed, however, that they had all previously suffered from malaria, and especially subtertian malaria, a disease from which probably no native child in the Gold Coast escapes. In the opinion of some authors, malarial endarteritis is a well-defined condition; Moreau (1918), for example, has recently referred to two cases of gangrene of the lower extremities, which he believed were the result of posterior tibial endarteritis, due to malaria. It is possible that the endarteritis in these cases of cardiac aneurysm was due to malaria, but further work on the pathology of the disease is necessary before this connexion can be established.

Obstruction of the arterioles is, however, admitted to be common in malaria, especially subtertian malaria, and to be the cause of many and varied complications. Dudgeon and Clarke (1917), in a recent study of the microscopical histology of malaria, remark that 'this vascular obstruction would seem to be most marked in the brain, spleen, and heart muscle.' The same authors found in all the six cases they examined fatty degeneration of the heart muscle, a condition which Legg considered 'certainly deserves attention as a cause of aneurysm.' If Hall is correct in attributing to coronary obstruction the chief part in the causation of aneurysms of the left ventricle, there seems no reason why such aneurysms should not be due to malaria.

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FIG. 1



FIG. 2



FIG. 3

THREE CASES OF CARDIAC ANEURYSM

STRONGYLIDÆ IN HORSES

IX. CYLICOSTOMUM TRIDENTATUM sp. n.

BY

WARRINGTON YORKE

AND

J. W. S. MACFIE

(Received for publication 13 August, 1920)

This worm was found in the caecum and colon of a young mule at Accra in the Gold Coast, West Africa, and in a horse in Jamaica.

SIZE AND SHAPE. A moderately small species of the Genus *Cylicostomum*. Eight females and eight males were measured. The males measured 7 mm. to 7.5 mm. in length, average 7.25 mm.; the females from 7.6 mm. to 9.75 mm., average 9.2 mm. The greatest breadth in the worms, when properly orientated, averaged in the males 307 μ , and in the females 450 μ .

HEAD. A well-marked neck separates the head from the body.

Mouth collar. Marked off from the rest of the skin by a definite constriction.

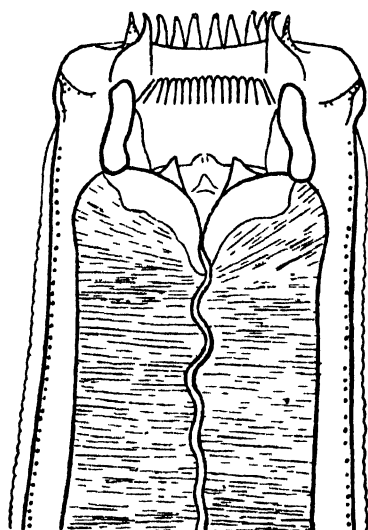
Head papillae. Sub-median, prominent conical and projecting, their extremities separated from the remainder by a constriction; lateral, scarcely projecting.

Mouth capsule. Almost circular in transverse section, the lateral diameter being usually slightly greater than the dorso-ventral diameter. When the worm is properly orientated the walls of the mouth capsule seen in optical section are stout, of approximately uniform thickness, and diverge slightly from before backwards. (fig. 1). When viewed laterally the walls of the buccal capsule have a similar appearance, and also diverge slightly from before backwards. In properly orientated worms the antero-posterior diameter (*i.e.*, the distance from the anterior to the posterior opening) of the buccal capsule varied in the males from 24 μ to 29 μ , average 26 μ , and in the females from 29 μ to 34 μ , average 32.3 μ . In the males the lateral diameter of the buccal capsule at the anterior

opening was 40μ to 41μ , and at the posterior opening 50μ ; in the females the lateral diameter of the buccal capsule at the anterior opening varied from 48μ to 54.5μ , average 52.6μ , and at the posterior opening from 55μ to 66.5μ , average 59.6μ . The ratio of the lateral diameter of the buccal capsule at the anterior opening to the antero-posterior diameter is in both sexes about 1.5 to 1.

Dorsal oesophageal gutter. Projects into the buccal capsule as a small tubercle.

Leaf crowns. The external leaf crown consists of about twenty large pointed elements arising from the mouth collar. The internal leaf crown consists of about thirty-six rather long, moderately broad elements, having a pallisade-like appearance arising in a single plane within the mouth capsule near its anterior opening (fig. 1).



A. M. B., del.

FIG. 1. *Cylicostomum tridentatum*, sp. n.
Anterior extremity, ventral view. $\times 360$.

OESOPHAGUS. The length in the males was 370μ to 384μ , average 376μ , and the greatest breadth 104μ to 115μ , average 109μ , the ratio of breadth to length was 1 to 3.5. In the females the length ranged from 445μ to 519μ , average 481μ , and the greatest breadth from 130μ to 133μ , average 131μ ; the ratio of breadth to length was 1 to 3.8. The ratio of the length of the oesophagus to that of

the worm was, in the male 1 to 19'2, and in the female 1 to 19'1. The chitinised oesophageal funnel is well developed; corresponding to the three divisions of the oesophagus are three small pointed

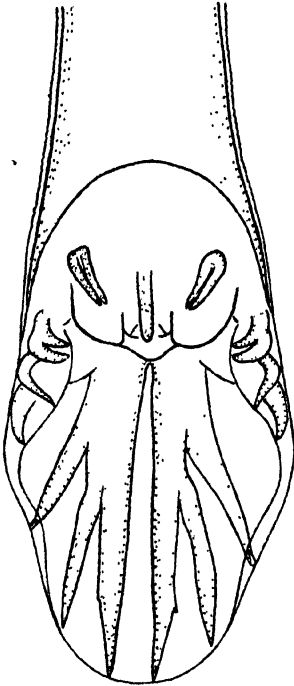


FIG. 2.



FIG. 3.

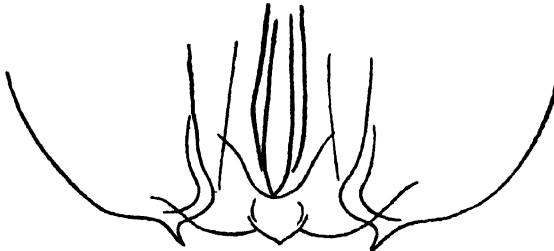


FIG. 4.

A. M. B., del.

FIGS. 2-4. *Cylicostomum tridentatum*, sp. n.

FIG. 2. Posterior extremity of male, ventral view. $\times 90$.

FIG. 3. Posterior extremity of male, lateral view. $\times 90$.

FIG. 4. Genital cone, ventral view. $\times 360$.

triangular teeth projecting from the oesophageal funnel into the mouth capsule, one dorsal and two ventro-lateral.

EXCRETORY BLADDER. Lies a little behind the nerve ring. The distance of its posterior margin from the posterior end of the oesophagus varied from 96μ to 140μ , average 114μ .

CERVICAL PAPILLAE. Lie at about the same level as the excretory bladder, or a little anterior to it.

POSTERIOR EXTREMITY OF MALE. The dorsal lobe of the bursa is moderately long (figs. 2 and 3). The length of the main trunk of the posterior ray, from the tip to the point of origin of the postero-external rays, was in the males 420μ to 495μ , average 447μ . The ratio of the length of the main trunk of the posterior ray to the length of the male worm was 1 to 16.

Genital cone. The dermal collar is well developed on both the ventral and dorsal surfaces of the genital cone. The genital appendages are absent. There were, however, two finger-like processes on the posterior part of the dermal collar similar to those in *C. labiatum* (fig. 4).

POSTERIOR EXTREMITY OF THE FEMALE. The end of the body was bent dorsally, usually only to a moderate extent, but in one specimen at a right angle. The ventral prominence was usually large and projecting. The tail was short and conical (fig. 5).

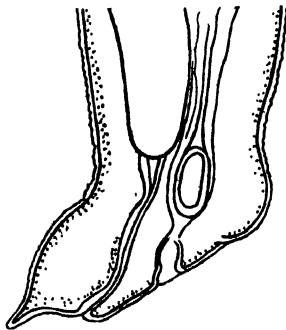


FIG. 5. *Cylicostomum tridentatum*, sp. n.

Posterior extremity of female, lateral view. $\times 90$.

The distance between the anus and vulva varied from 102μ to 151μ , average 118μ , and the distance measured straight along the middle line of the tail, from the tip to a line drawn horizontally through the anus, varied from 95μ to 108μ , average 100μ .

DIAGNOSIS. The following are the chief diagnostic characters of the worm :—

1. Size, moderately small; length, male 7·25 mm. and female 9·2 mm.
2. Buccal capsule: anterior opening almost circular; walls when seen in optical section, in properly orientated worms, stout, approximately uniform in thickness, and diverging slightly from before backwards; the ratio of breadth at anterior opening to antero-posterior diameter 1·5 to 1.
3. Dorsal oesophageal gutter projects as a tubercle.
4. Arising from the oesophageal funnel are three small pointed triangular teeth, one dorsal and two ventro-lateral.
5. Dorsal lobe of bursa moderately long; ratio of length of posterior ray to total length of male worm 1 to 16. The genital appendages are absent; there are, however, two finger-like processes on the posterior part of the dermal collar.
6. Termination of body of female bent dorsally, generally slightly but occasionally at a right angle. Ventral prominence large. Tail short and conical.

This species belongs to the *catinatum-alveatum* group of Cylicostomes. It most closely resembles *C. alveatum*, but may be distinguished from it by its smaller size, the almost circular anterior opening of the mouth capsule, the projection of the dorsal oesophageal gutter as a tubercle, and by the three small teeth arising from the oesophageal funnel. We propose for this species the name *Cylicostomum tridentatum*.

STRONGYLIDAE IN HORSES

X. ON THE GENUS *POTERIOSTOMUM*, Quiel

BY

WARRINGTON YORKE

AND

J. W. S. MACFIE

(Received for publication 2 September, 1920)

In 1919, Quiel described a Strongyle (*Poteriostomum imparidentatum*) found in the caecum and colon of horses at Donchery, which, although closely resembling a Cylicostome, differed from members of this genus in certain respects. For this worm he erected the genus *Poteriostomum*, which he defined as follows:— A sclerostome genus with a well developed internal leaf crown, most closely related to the genus *Cylichnostomum*, Looss, but distinguishable from the latter by the fact that the six leaves of the inner leaf crown lying in the radii of the head papillae are differentiated from the others by their greater length and by their distinct shape, and by the fact that there are no bosses at the posterior end of the female. Quiel called this worm *P. imparidentatum*. A little later the same worm was discovered independently in Dutch horses by Ihle (1920), who also created a new genus for it and gave it the name *Hexodontostomum markusi*, and in a Chapman's zebra by Turner (1920) who called it *Cylichnostomum zebrae*.

Shortly before the appearance of Quiel's paper, Kotlán had

described a Strongyle found in Hungarian horses which bears a close resemblance to Quiel's *Poteriostomum*, but differs from it in that all the elements of the internal leaf crown are of the same size. To this parasite Kotlán gave the name *Cylichnostomum ratsii*. This worm was subsequently found by Ihle (1920) in Dutch horses.

Both the above worms were found by us in material obtained from a young mule at Accra in the Gold Coast. On comparing them we were at once impressed with their close resemblance; *C. ratsii* differing from *P. imparidentatum* chiefly by the character of the internal leaf crown, the elements of which were in *C. ratsii* of equal size and furnished with pointed tips, and in *P. imparidentatum* of unequal size (those corresponding to the head papillae being longer and pointed) and having rounded ends.

We were particularly impressed by the fact that the bursae were similar and differed markedly from those found in any hitherto described species of the genus *Cylicostomum*. Ihle has given an admirable description of this structure; he points out that the supporting rays form two distinct groups, an antero-median and a posterior. It is in the character of the posterior group that the bursa chiefly differs from that of *Cylicostomes*; the postero-external ray arises from a common trunk with the posterior, the main trunk of the posterior ray is not split to the base, but only for about half its length, the two lateral branches arising from the undivided portion close to the point of origin of the postero-external rays. These characters are clearly shown in figs. 1 to 6.

This arrangement of the posterior rays of the bursa appears to us to warrant the separation of both the worms from the genus *Cylicostomum*; whereas the slight difference in the characters of the internal leaf crown of the two worms, which is much less than that exhibited by such *Cylicostomes* as *C. radiatum*, Looss, and *C. bicoronatum*, Looss, does not, in our opinion, justify placing them in different genera. We, therefore, propose to regard both worms as belonging to the genus *Poteriostomum*, and suggest that the definition of the genus be emended to include all *Cylicostome*-like worms having the bursal characters described above.

Accepting this definition of the genus, the following worms must then be included in it:—

Poteriostomum imparidentatum, Quiel, 1919 (syn. *Hexodontostomum markusi*, Ihle, 1920, and *Cylichnostomum zebrae*, Turner, 1920.

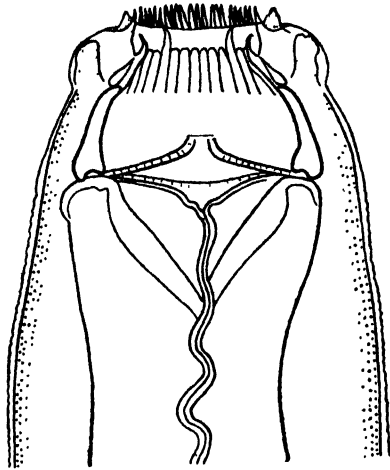


FIG. 1.

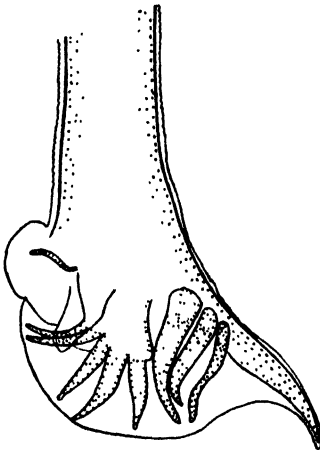


FIG. 2.

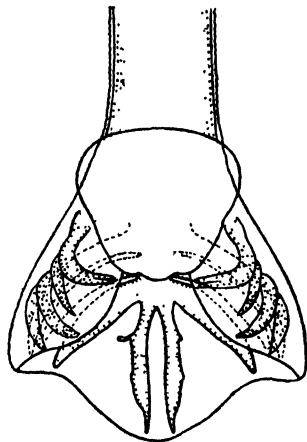


FIG. 3.

A. M. B., del.

Poteriostomum imparidentatum, Quiel.

FIG. 1. Anterior extremity, ventral view. $\times 180$.

FIG. 2. Posterior extremity of male, lateral view. $\times 45$.

FIG. 3. Posterior extremity of male, ventral view. $\times 45$.

Poteriostomum pluridentatum, Quiel, 1919.

Poteriostomum ratzii (Kotlán), 1919. (syn. *Cylichnostomum ratzii*, Kotlán, 1919.

Perhaps *Cylicostomum ultrajectimum*, Ihle, 1920, also belongs this genus, but as only a single female has so far been found, it is impossible to say.

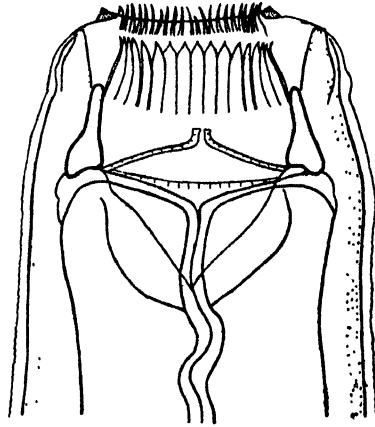


FIG. 4.

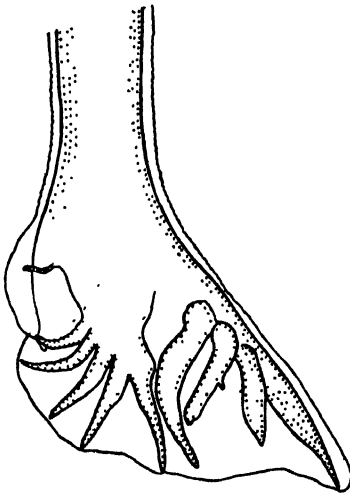


FIG. 5.

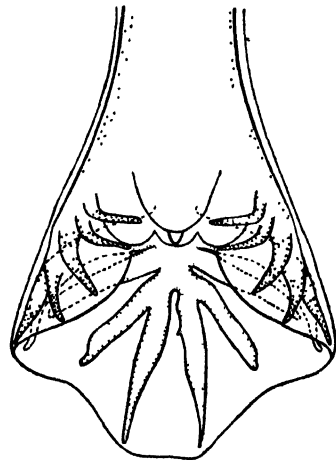


FIG. 6.

A. M. B., del.

Poteriosomum ratzii, (Kotlán).

FIG. 4. Anterior extremity, ventral view. $\times 180$.

FIG. 5. Posterior extremity of male, lateral view. $\times 45$.

FIG. 6. Posterior extremity of male, ventral view. $\times 45$.

The species of the Genus *Poteriosomum* hitherto described may be distinguished as follows:—

1. All the elements of the internal leaf crown of
equal length *P. ratzii*.
Elements of the internal leaf crown not all of
equal length 2
2. Internal leaf crown with seven normal elements
between the longer ones ... *P. imparidentatum*.
Internal leaf crown with ten normal elements
between the longer ones ... *P. pluridentatum*.

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STRONGYLIDAE IN HORSES

XI. SPECIES FOUND IN WEST AFRICA AND JAMAICA

BY

WARRINGTON YORKE

AND

J. W. S. MACFIE

(Received for publication 2 September, 1920)

Species found in West Africa

The material to which this note relates was obtained from three horses and one young mule bred in West Africa; one of the horses was examined at Ilorin in Nigeria, and one at Tamale in the Northern Territories of the Gold Coast, and the other two animals at Accra. We are indebted to Dr. K. B. Allan for very kindly sending us the specimens from Tamale.

The horse and the young mule examined at Accra had been condemned to be shot because of their poor condition. They were both very weak, greatly emaciated, and apparently dying. At the post-mortem examinations, which were made by one of us, numerous *Strongylidae* were found in the caecum and colon of both animals, and in the young mule particularly there were vast numbers of them. No other cause was found to account for the sickness of the animals, blood examination was negative, and it was therefore concluded that their condition was due to infection with these worms. The horse examined at Ilorin had died of trypanosomiasis.

In the following table a list is given of the species of *Strongylidae* found in each animal.

| | Young Mule : Accra, Gold Coast. | Horse : Accra, Gold Coast. | Horse : Tamale, Gold Coast. | Horse : Ilorin, Nigeria. |
|---|---|-------------------------------------|--------------------------------------|--------------------------------|
| <i>Strongylus.</i> | | | | |
| <i>S. edentatus</i> , Muller, 1784 | + | + | + | + |
| <i>S. equinus</i> , (Looss), 1900 | ... | + | ... | ... |
| <i>S. vulgaris</i> , (Looss), 1900 | + | + | ... | + |
| <i>Triodontophorus</i> | | | | |
| <i>T. intermedius</i> , Sweet, 1908 | + | + | ... | ... |
| <i>T. minor</i> , Looss, 1900 | + | + | ... | ... |
| <i>Cylicostomum</i> | | | | |
| <i>C. alveatum</i> , Looss, 1900 | ... | ... | ... | + |
| <i>C. catinatum</i> var. <i>litoraureum</i> , var. n. | ... | ... | + | ... |
| <i>C. coronatum</i> , Looss, 1900 | + | ... | + | + |
| <i>C. insigne</i> , Boulenger, 1917 | + | ... | ... | ... |
| <i>C. labiatum</i> , Looss, 1901 | + | ... | ... | ... |
| <i>C. longibursatum</i> , Yorke and Macfie, 1918 | + | ... | ... | ... |
| <i>C. nassatum</i> , Looss, 1900 | ... | ... | + | + |
| <i>C. nassatum</i> var. <i>parvum</i> , Yorke and Macfie, 1918 | + | ... | ... | ... |
| <i>C. poculatum</i> , Looss, 1900 | + | ... | ... | ... |
| <i>C. pseudo-catinatum</i> , Yorke and Macfie, 1919 | + | ... | ... | ... |
| <i>C. radiatum</i> , Looss, 1900 | + | ... | ... | ... |
| <i>C. tridentatum</i> , Yorke and Macfie, 1920 | + | ... | ... | ... |
| <i>Poteriostomum</i> | | | | |
| <i>P. imparidentatum</i> , Quiel, 1919 | + | ... | ... | ... |
| <i>P. ratzii</i> , (Kotlán), 1919 | + | ... | ... | ... |

One of the above species requires special mention, viz. :—

Cylicostomum catinatum var. *litoraureum*, var. n.

This worm was obtained from the caecum and colon of a horse

at Tamale in the Northern Territories of the Gold Coast, West Africa.

One male and seven females were found. The length of the male was 9 mm.; the lengths of the females ranged from 8.75 mm. to 12 mm., average 9.9 mm.

The worm was indistinguishable from *C. catinatum*, Looss, excepting by the characters of the appendages of the genital cone, which exhibited a striking difference. As is shown in fig. 2, the

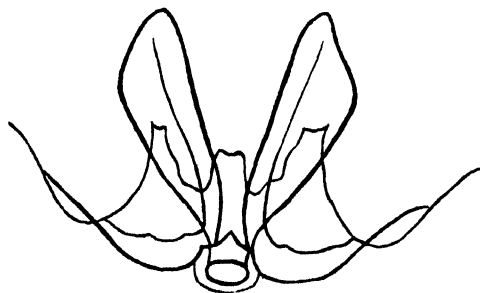


FIG. 1.

FIG. 1. *Cylicostomum catinatum*, Looss.

Genital appendages, ventral view (after Looss). $\times 360$.



A. M. B., del.

FIG. 2.

FIG. 2. *Cylicostomum catinatum* var. *litoreaureum*, var. n.

Genital appendages, ventral view. $\times 360$.

appendages of this worm consisted of two long finger-like processes, each bearing a tubercle on its inner aspect and a short process between them, whereas, according to Looss, the appendages of the genital cone of *C. catinatum* consist of 'medium long processes with notched outlines and rounded extremities.'

We regard this worm as a variety of *C. catinatum*, Looss, and propose to call it *Cylicostomum catinatum* var. *litoreaureum*.

Species found in Jamaica

Material from one horse was examined, and the following species identified:—*C. euproctus*, *C. longibursatum*, *C. nassatum* var. *parvum*, and *C. tridentatum*.

STRONGYLIDAE IN HORSES

XII. *CYLINDROPHARYNX RHODESIENSIS* sp. n.

BY

WARRINGTON YORKE

AND

J. W. S. MACFIE

(Received for publication 20 September, 1920)

SIZE AND SHAPE. A large species of the Genus *Cylindropharynx*. One male and four females were found in the large intestine of a zebra (*Equus burchelli*) shot in Northern Rhodesia. The male measured 12·5 mm. in length and its greatest breadth was about 650 μ . The females varied in length from 13·5 to 15·8 mm., average 14·6 mm.; their greatest breadth was about 830 μ .

HEAD. *Mouth collar.* Marked off from the rest of the skin by a constriction; it is distinctly higher dorsally and ventrally than laterally.

Head papillae. Sub-median, large and prominent, the distal, spindle shaped, portion being separated off from the proximal, conical, portion by a constriction. Lateral, not conspicuous, scarcely projecting.

Mouth capsule. Exhibits the great length characteristic of the Genus (fig. 1). It is almost circular in transverse section, the dorso-ventral diameter being slightly greater than the lateral. The antero-posterior diameter was in the male 520 μ , and in the females varied from 511 μ to 544 μ , average 526 μ . The greatest dorso-ventral diameter was in the male 144 μ , and in the females varied from 175 μ to 178 μ , average 177 μ . The greatest lateral diameter was in the male 138 μ , and in the females varied from 161 μ to 165 μ , average 164 μ . The ratio of the greatest breadth to the length of the mouth capsule was in the male 1 to 3·8, and in the females 1 to 3·2. The

walls of the mouth capsule are stout, of almost uniform thickness except at the two extremities, where they taper slightly: they are almost parallel, the greatest diameter is found near the anterior opening (figs. 1 and 2).

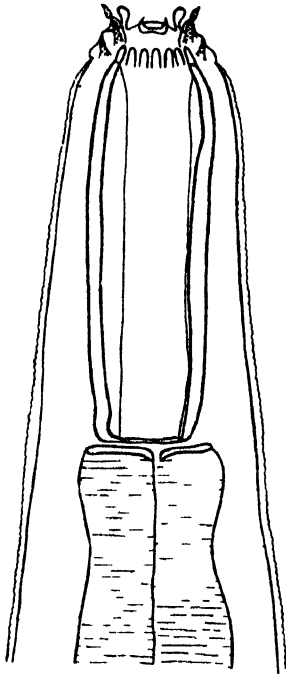


FIG. 1.

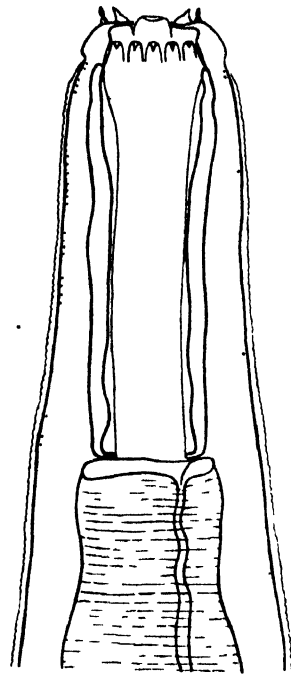


FIG. 2.

A. M. B., del.

Cyliodropharynx rhodesiensis, sp. n.

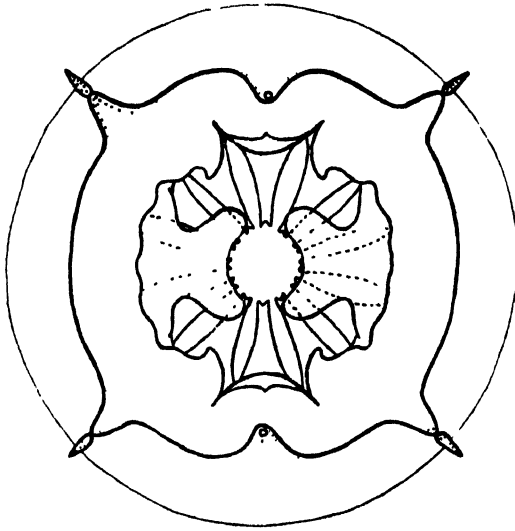
FIG. 1. Anterior extremity, ventral view. $\times 90$.

FIG. 2. Anterior extremity, lateral view. $\times 90$.

Dorsal oesophageal gutter. Does not project into the buccal capsule.

Leaf crowns. The external leaf crown consists of six large teeth corresponding in position to the head papillae. The four occupying the sub-median positions are somewhat smaller than the two situated laterally, which are also much broader. Dorsally and ventrally the external leaf crown is deficient, but from each of the prominent dorsal and ventral lips of the mouth collar there projects horizontally inwards a broad crescentic plate, the free, concave

margin of which is directed towards the axis of the mouth (see fig. 3). The internal leaf crown arises from the anterior extremity of the mouth capsule. It consists of twelve large teeth.



Cylindropharynx rhodesiensis, sp. n.

FIG. 3. End on view of anterior extremity. $\times 360$.

OESOPHAGUS. The oesophagus is short and broad. The nerve ring is situated near the anterior extremity. The length was in the male 767μ , and in the females varied from 833μ to 841μ , average 838μ .

EXCRETORY BLADDER. Lies near the posterior end of the oesophagus.

CERVICAL PAPILLAE. Lie in front of the excretory bladder, about half-way between the nerve ring and the posterior end of the oesophagus.

POSTERIOR EXTREMITY OF THE MALE. The dorsal lobe of the bursa is short. The posterior ray exhibits the following characters: it is split to its base, and from each limb arises one lateral branch close to the point of origin of the postero-external ray, the extremity of the lateral branch bifurcates into two finger-like processes, the

external of which is slightly the longer. The main trunks of the posterior ray taper to very fine points (fig 4).

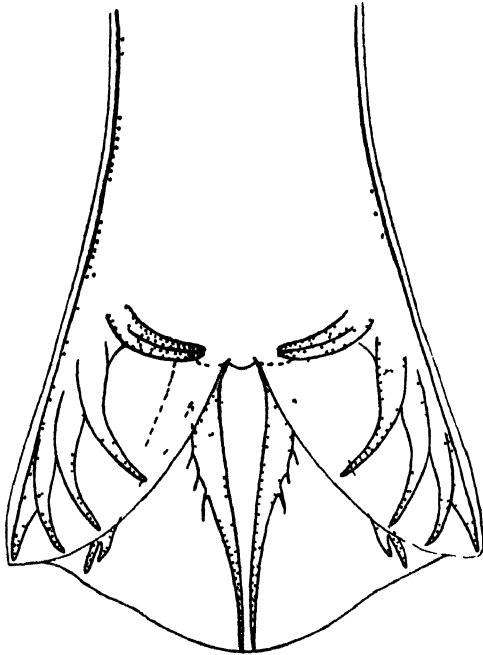


FIG 4.



FIG 5



FIG 6.

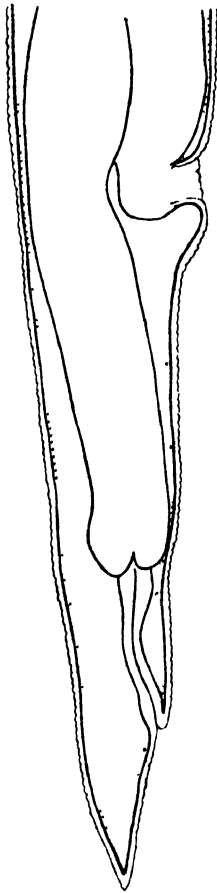
Cylindropharynx rhodesiensis, sp n

- FIG 4 Posterior extremity of male, ventral view $\times 90$.
 FIG 5 Spicules, ventral view $\times 360$
 FIG 6 Spicules, lateral view $\times 360$

Genital cone. The genital cone is globular. The dermal collar surrounds the cone, but is especially well developed on the ventral surface. The genital appendages appear to be absent. The spicules are long, stout and barbed, as shown in figs. 5 and 6.

POSTERIOR EXTREMITY OF THE FEMALE The end of the body is

straight and gradually tapers to a point (fig. 7). The distance from the anus to the tip of the tail varies from 388μ to 480μ , average 417μ ; and that from the vulva to the tip of the tail from $1,700\mu$ to $2,196\mu$, average $1,897\mu$.



A M B, del

Cylindropharynx rhodesiensis, sp. n.

FIG. 7. Posterior extremity of female. $\times 45$.

DIAGNOSIS. This worm differs from the other two known species of the genus by its much greater size, and may be further distinguished by the large size of its mouth capsule and by certain details of the posterior ends of both male and female. We propose for this worm the name *Cylindropharynx rhodesiensis*. For purposes of comparison these differences are shown in the table.

| | <i>Cylindrobarynx rhodesiensis</i> Yorke and Macfie, 1920 | <i>Cylindrobarynx brevicauda</i> Leiper, 1911 | <i>Cylindrobarynx longicauda</i> Leiper, 1911 |
|--|--|--|--|
| 1. Length, ♂ | 12.5 mm. | 5-7.3 mm. | 4.7-5.8 mm. |
| ♀ | 13.5-15.8 mm. | 5.6-8 mm. | 6.2-7 mm. |
| 2. Mouth capsule | Antero-posterior diameter 511-544 μ , Lateral diameter 138-165 μ | Antero-posterior diameter 300-400 μ , Lateral diameter 90-120 μ | Antero-posterior diameter 180-200 μ , Lateral diameter 70-90 μ |
| 3. Elements of the leaf crowns : | | | |
| External | — | — | — |
| Internal | — | — | — |
| 4. Oesophagus | Length : ♂ 767, ♀ 833 to 841 μ | Length : 470-530 μ | Length : 420-450 |
| 5. Posterior extremity of male : | Extremity of external branch bifurcated | Extremity of external branch bifurcated | Extremity of external branch undivided or with only a minute process |
| Posterior ray | | | |
| Genital cone | Shorter and more globular | Almost cylindrical, completely surrounded by dermal collar | Shorter and more globular |
| Genital appendages ... | Apparently absent | A pair of finger-shaped append- ages with rounded ends and behind them a number of delicate pointed processes | A single pair of rather stout finger-shaped processes |
| 6. Posterior extremity of female : | | | |
| Distance of vulva from tip of tail | 1700-2196 μ | 450-750 μ | 1100-1200 μ (Leiper 1550 μ) |
| Distance of anus from tip of tail | 388-480 μ | 150-200 μ | 280 μ (Leiper 320 μ) |

Leiper (1911), who described the Genus, and also the two species *C. brevicauda* and *C. longicauda*, omitted to give a full description of certain important structures, *e.g.*, the oral leaf crowns and the genitalia of the males. Boulenger (1920), in a recent paper, has supplied information on the points in which Leiper's description was deficient. Boulenger, however, failed to draw attention to the horizontal plates which we have found in *C. rhodesiensis* to project from the dorsal and ventral lips of the mouth collar. These plates are not peculiar to *C. rhodesiensis*, but were also seen by us in *C. brevicauda*, and it is probable that they are a generic character.

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STRONGYLIDÆ IN HORSES

XIII. CYLICOSTOMUM TRIRAMOSUM sp. n.

BY

WARRINGTON YORKE

AND

J. W. S. MACFIE

(Received for publication 20 September, 1920)

This worm was found in the intestine of a zebra (*Equus burchelli*) shot in Northern Rhodesia. One male and one female were found.

SIZE AND SHAPE. A moderate sized species of the genus *Cylicostomum*. The male and female each measured 12·3 mm. in length. The greatest breadth was in the male about 630 μ , and in the female about 750 μ .

HEAD. *Mouth collar.* Marked off from the skin by a slight constriction; moderately high and voluminous.

Head papillae. Sub-median, prominent, their extremities separated from the proximal portions by a constriction; lateral, prominent and projecting as slight horns.

Mouth capsule. Ellipsoidal in transverse section, the ratio of the lateral diameter to the dorso ventral diameter at the anterior opening of the buccal capsule being as 1·2 to 1. When the worm is properly orientated the walls of the mouth capsule seen in optical section are straight, moderately slender anteriorly and stouter with a hoop-like thickening posteriorly (fig. 1). In the properly orientated worm the antero-posterior diameter (*i.e.*, the distance from the anterior to the posterior opening) of the buccal capsule was 38 μ . The lateral diameter of the buccal capsule at the anterior opening was 110 μ , and the dorso-ventral diameter 90 μ . The ratio of the lateral diameter of the buccal capsule at the anterior opening to the antero-posterior diameter is about 2·75 to 1.

Dorsal oesophageal gutter. Does not project into the buccal capsule.

Leaf crowns. The external leaf crown consists of at least thirty large pointed elements arising from the mouth collar. The internal leaf crown consists of numerous minute elements arising from the anterior margin of the mouth capsule.

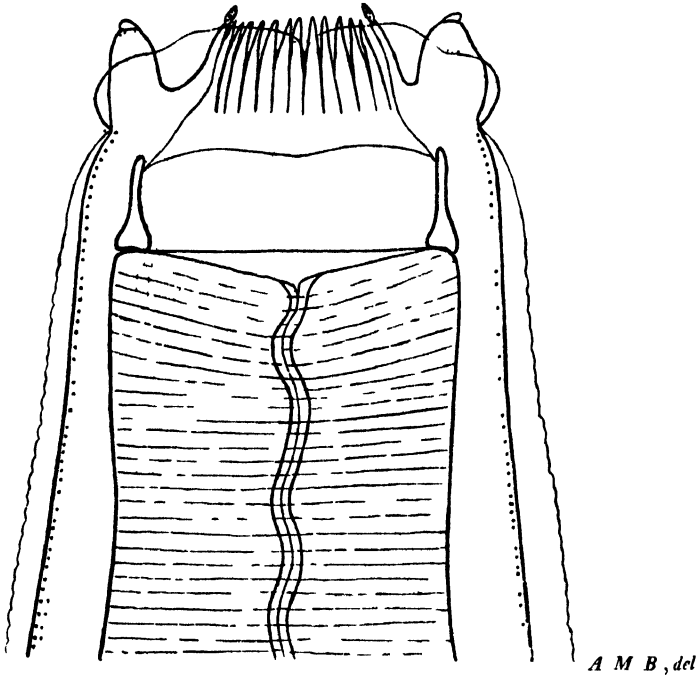


FIG. 1. *Cylicostomum tiramosum*, sp. n.
Anterior extremity, ventral view. $\times 360$.

OESOPHAGUS. The length in the male was 840μ , and in the female 940μ . The ratio of the length of the oesophagus to that of the worm is in the male 1 to 14.6, and in the female 1 to 13.1.

EXCRETORY BLADDER. Lies just behind the nerve ring.

CERVICAL PAPILLAE. Lie at about the same level as the excretory bladder.

POSTERIOR EXTREMITY OF THE MALE. The dorsal lobe of the bursa is of moderate length. The length of the main trunk of the posterior ray, from the tip to the point of origin of the postero-external rays,

was 635μ . The ratio of the length of the main trunk of the posterior ray to the length of the male worm is as 1 to 20. The posterior ray gives off three lateral branches, the upper two of which arise close together (figs. 2 and 3).

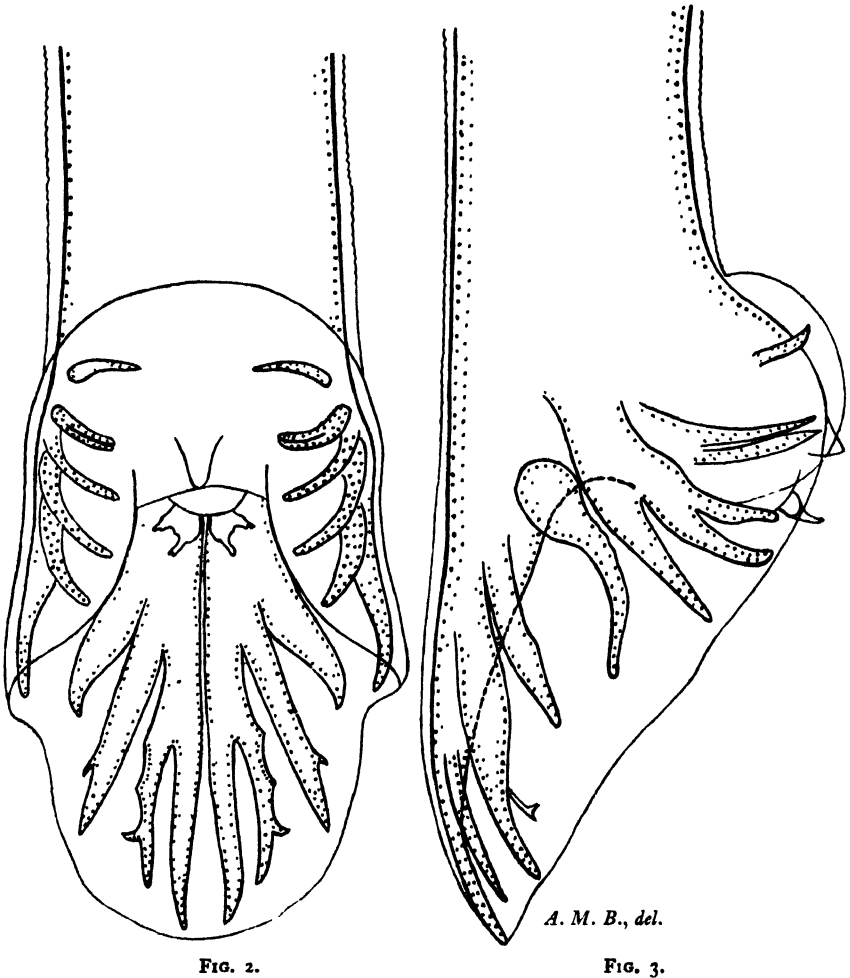


FIG. 2.

FIG. 3.

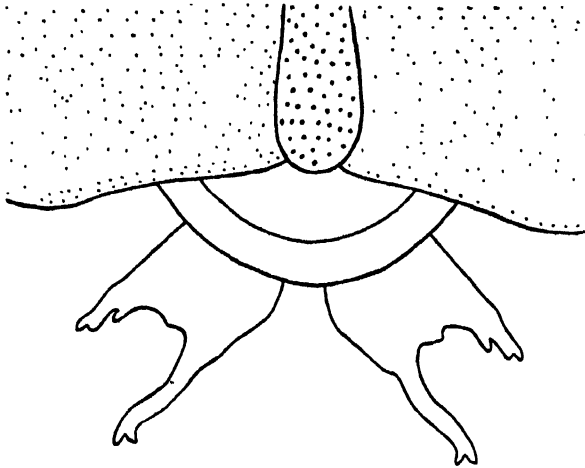
FIGS. 2-3. *Cylicostomum triramosum*, sp. n.

FIG. 2. Posterior extremity of male, ventral view. $\times 90$.

FIG. 3. Posterior extremity of male, lateral view. $\times 90$.

Genital cone. The dermal collar is well developed on both the ventral and dorsal surfaces of the genital cone. The genital appendages consist of two large plates arising separately, diverging

from the middle line, and each terminating in two finger-like processes, the inner of which is the larger. The finger-like processes are bifid at their tips (fig. 4).



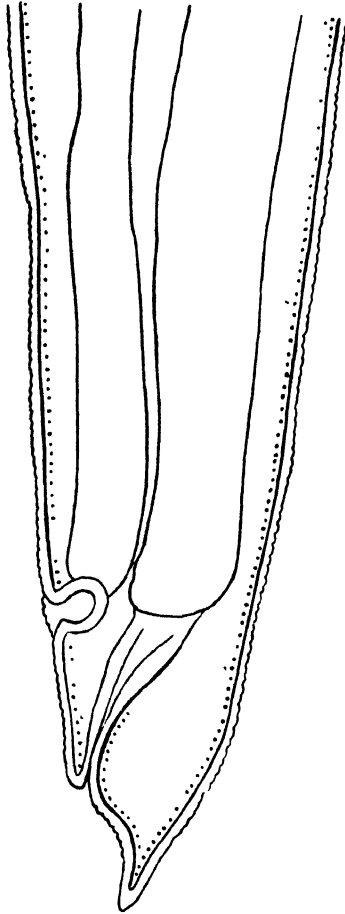
A. M. B., del.

FIG. 4. *Cyclostomum triramosum*, sp. n.

Genital cone, ventral view. $\times 360$.

POSTERIOR EXTREMITY OF THE FEMALE. The end of the body is straight. The tail is short and conical (fig. 5). The distance between the anus and the vulva was 264μ , and the distance measured straight along the middle line of the tail from the tip to a line drawn horizontally through the anus was 168μ .

DIAGNOSIS. This worm resembles *Cyclostomum radiatum*, Looss, most closely, but may be distinguished from it by the prominence of the lateral papillae of the mouth collar, by the posterior ray of the bursa bearing three branches, and by the characteristics of the genital appendages of the male.



A. M. B., del.

FIG. 5 *Cylicostomum triramosum*, sp. n.
Posterior extremity of female, lateral view. $\times 90$.

FISH AND MOSQUITO LARVAE IN BENGAL, BIHAR AND ORISSA, INDIA

BY

T. SOUTHWELL

(Received for publication 21 June, 1920)

Most people interested in malaria are aware of the fact that in India, and elsewhere, certain species of fish feed extensively on mosquito larvae.

We have much to learn yet concerning the habits of many of the above species of fish, their relation to other species, and the conditions under which they act effectively, before one can assess, approximately, their value as mosquito-reducing agents. In India, at least, it is only within the last ten years that the subject has received attention. The present paper summarises, roughly, information obtained through the investigations carried out by Sewell, Chaudhuri, Alcock, Bentley and Lloyd. In addition, it affords the writer an opportunity of recording, for the first time, impressions and conclusions arrived at as a result of eight years' work in India as Director of Fisheries to the Governments of Bengal and Bihar and Orissa.

Monsoon. During the early part of June, the south-west monsoon-rains commence, and, in a short time the province is more or less flooded. It is important to note that the breeding season for many species of fish coincides with the advent of the monsoon. Consequently fishes' eggs and young fish are, at this time, caught in large numbers and are found in almost every ditch and pool of water. By the middle of August, the rains are practically over and the dry season commences, continuing to the following June. The flooded areas contract, and eventually become dry; the young fish so lavishly distributed in nature become isolated in little pools and are either caught or die, except those which are successful in reaching a tank, beel or river.

Water areas. (1) *Rivers and irrigation canals.* Bengal is a low-lying country, rich in waterways. The main river, the Ganges, with its numerous tributaries, drains the province and, a little to the South of Calcutta, breaks up into the Gangetic Delta. The latter area, which is known as the Sunderbans, comprises an immense number of swift-flowing tidal streams, intersecting each other in all directions, together with swamps and dense virgin jungle. Irrigation is carried on throughout the province by means of a system of canals which arise from both sides of anicuts, or weirs, thrown across rivers. They distribute water, during the dry season, to the paddy or rice fields.

(2) *Beels.* Beels are also numerous; these are large *natural* depressions, full of water, corresponding to our lakes, for the most part retaining a connection with a river, either throughout the year or during the rains only. They, too, contain fish in considerable abundance ..

(3) *Borrow pits.* Borrow pits occur 'pitifully'. They measure two or three yards long, two or three feet wide and a foot or so deep, and are formed as a result of earth having been removed in order to elevate surrounding land, usually railway embankments.

(4) *Tanks.* Tanks (or ponds) in Bengal play a very important and extensive rôle in the economy of village life; they are utilised by the villagers for bathing, washing and for a variety of other purposes. They are exceedingly numerous. They were dug originally either because earth was necessary to elevate the ground on which a house was to be built, above the flood level, or from religious motives. In years gone by, tanks were kept in much better repair than is the case nowadays, owing, probably, to the fact that the growth and development of western education and commerce has attracted village life to the cities and towns.

Many tanks have become more or less silted up, foul, or choked with weed. The result is that they form admirable breeding-places for certain species of mosquitoes.

A considerable number of species of fish occur in tanks. The large and edible species include carps like *Labeo rohita*, and cat-fishes such as *Wallago attu*, *Macrones* spp., etc., but other smaller species growing to two or three inches in length are numerous. In most tanks there are found, as a rule, only a very few large fishes,

such as carps or cat-fishes, the former being individuals which have escaped the ravages of predatory species. Such small species as do occur are found, as a rule, towards the edges of the pool. The tanks in which predatory fish do *not* occur form a very small percentage. These facts are of importance in that any scheme for stocking tanks, beels, streams, etc., with mosquito larvae-eating fish which takes no account of the presence of predatory species, is doomed to failure.

Mosquito larvae-eating fish. There exists in Barbadoes a fish known as 'Millions' (*Poecilia poeciloides*, De Filippe, = *Girardinus poeciloides*), which is well known as being very destructive to mosquito larvae. It was introduced into Ceylon for that purpose about twenty years ago. It has since practically died out in the Island. It was also introduced into India by the Government of the United Provinces a little later, and a number of specimens were sent to the tanks in the Zoological Gardens in Calcutta. Apparently they did not thrive and increase. It is certain, however, that in Bengal there is at least one species (*Haplochilus panchax*, Hamilton Buchanan = *Panchax panchax*, Tate Regan) which is superior to 'Millions' as a destroyer of mosquito larvae. The species of fish best adapted for destroying mosquito larvae should breed in confined areas, otherwise they will die unless replenished year by year.

The following species of fishes occur abundantly in fresh water in India, and all are known to eat mosquito larvae extensively under natural conditions:—

Genus *Haplochilus*.

Species of the genus *Haplochilus* are small, and the adult seldom grows to three inches in length. This genus belongs to the group *Carnivorae* of the family *Cyprinodontidae* and to the sub-order *Haplomi*.

There are three species:—

1. *Haplochilus panchax* (Hamilton Buchanan). Occurs all over India. It has a flat head and lives on the surface of the water.

2. *Haplochilus melastigma* (McClell). Smaller than *panchax*. Widely distributed throughout India.

3. *Haplochilus lineolatus* (Cuv. and Val.). Grows to four inches and occurs plentifully.

All the above three species are hardy, and breed freely in confined water. Mosquito larvae are to be found normally on the surface of the water, and these fishes, which also live on the surface, devour them in nature with exceptional avidity.

Species of less importance are:—

Ambassis nama (Ham. Buch.). Two to three inches long; breeds freely in confined water, occurs abundantly.

A. ranga is smaller than the above, but similar in its habits. They are not surface feeders.

Trichogaster fasciatus (Bl. Schn.). Grows to four or five inches in length and occurs in fresh and brackish water. It has a wide distribution in India.

Badis badis (Ham. Buch.). Lives in mud and is very voracious. Occurs both in fresh and brackish water. Grows to four or five inches in length.

Barbus phutunio (Ham. Buch.). One to two inches long, and breeds in tanks. Of this genus there are at least three other species of some use as mosquito larvae destroyers, viz., *B. ticto*, *B. stigma* and *B. terio*. Some of the species have, however, a restricted distribution.

Anabas scandens (Daldorf). This species is known as the climbing perch. It possesses a respiratory system other than gills. It is able to climb small trees, and can live for a very long time entirely out of water. During the dry season, when tanks and other water areas dry up, this species buries itself in the damp mud and is actually dug up with a spade by the villagers. I have seen it taken from a depth of two feet from the surface of a dried-up pond. It grows to about eight inches in length and breeds freely in beels and even in brackish water; but it is doubtful whether it breeds in tanks, although, during the rains, its eggs and young find their way into tanks in large numbers like those of other species of fish.

Certain species of cat-fish (e.g., *Wallago attu*) found in tanks also have this habit of burying themselves in the mud during the dry weather, and the impossibility of removing them from tanks—especially if they breed there—will be obvious.

There are other species (such as *Perilampus* spp., *Danio rerio*, *Barilius* spp., *Rasbora daniconius*, etc.) which are also known to feed on mosquito larvae occasionally, and it is probable that still

other species will be discovered when investigations have been extended.

We may now consider briefly the question of the reduction of mosquitoes in nature on a large scale. At the present time the stocking of tanks with fish which devour mosquito larvae is frequently advocated as a means of mosquito reduction, and it is certain that the efficiency of this method is not doubted by many. It is true that the process may be considered a slow one, but in India time has but little value.

In connection with the problem, there are a few points which call for special consideration.

It is impossible to keep tanks, beels and other waterways stocked with mosquito larvae-eating fish, owing to the fact that they are themselves devoured by other and larger predatory fish. In order to remove the large predatory species it would be necessary to pump the water areas dry, to allow them to remain dry in the sun for months, and even then one could not be certain that the species buried in the mud might not spring to life again with the first rains. Further, it would be necessary to prevent the entrance of fresh broods of predatory fish during the next rains. Now such extensive preventive measures are, to my mind, clearly beyond the range of possibility. There is the expense, the enormous number of water areas to be dealt with, and the entire absence of any organisation to undertake the work. It will be obvious, I think, that the small species of fish which normally devour mosquito larvae have only a very limited usefulness in nature, and that this sphere of usefulness cannot be extended materially; as a result, the actual reduction of mosquitoes through the agency of fish is not at present a practical scheme. It is, however, impossible to say to what degree these fishes are useful, but as these agencies have been at work for some thousands of years, we may safely conclude that the effect is negligible.

In Bengal, tanks are naturally stocked with fish during every monsoon, and they, therefore, contain the maximum quantity of fish they are able to support. The artificial introduction of large numbers of mosquito larvae-eating fish upsets the established balance, which is ultimately righted by the death of those unable

to procure sustenance or which succumb to the predatory habits of other species in the tank.

The cultivation of carp along with mosquito larvae-eating fish is sound, because carp are not predatory, and there can be no doubt but that some slight improvement would result if more care was taken to stop the careless and ceaseless introduction of predatory species into tanks. This could be effected by stocking tanks with pure fry only, instead of with mixed species.

Lastly, attention may be called to the suggestion made of attacking the problem in India in a different way. It is known that if a little oil is placed in a pond it spreads and forms a delicate film on the surface of the water. This prevents mosquito larvae from obtaining sufficient air, and thus tends to kill them. As, however, it also tends to kill small fishes, and renders the water objectionable for domestic purposes, this procedure is not considered desirable by the village population.

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OBSERVATIONS ON THE CERATOPOGONINE MIDGES OF THE GOLD COAST WITH DESCRIPTIONS OF NEW SPECIES

PART I.

BY

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AND

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PLATES IV, V, VI

INTRODUCTORY

Small biting midges, colloquially referred to as "sand-flies," are abundant in the Gold Coast, and in many parts of the country are a vexatious pest. Very little appears to be known about them, however, only a few species have hitherto been described, and few, if any, observations have been made on their early stages and their breeding-places in this part of West Africa.

In December, 1919, a study of these midges was begun by two of us (J W S M and A I) at Accra. It was soon apparent that a large number and a great variety of specimens could be obtained, and that they would include a number of new species. The investigation is still in progress, but we propose to give in this and three or four succeeding papers an account—mainly descriptive—of the species, particularly those referable to the genera *Culicoides*, *Dasyhelea*, and *Forcipomyia*, obtained from December, 1919, to the beginning of May, 1920.

Most of the specimens were collected actually at Accra. Some, however, were procured in neighbouring towns and villages, and as the names of these places will have to be mentioned repeatedly in connection with the habitats of various species, it will be convenient here to explain where they are situated. The following are the places from which specimens were obtained :—

Accra : the capital of the Gold Coast Colony, a town situated on the sea-coast in a somewhat arid locality.

Oblogo : a village a few miles to the west of Accra, situated on the river Densu.

Odorkor : a small village between Accra and Oblogo.

Dodowah : a town about twenty-five miles north-east of Accra.

Adawso : a town about thirty miles north of Accra.

Nsawam : a town about twenty-five miles north of Accra, on the edge of the thick forest zone, and situated on the river Densu.

Koforidua : a town about fifty miles north of Accra.

Tafo : a town a little to the north-west of Koforidua.

Winnebah : a town on the coast a little to the west of Accra.

We have pleasure in acknowledging our indebtedness to Dr. F. H. Storey and Dr. D. Duff for specimens from the Koforidua district and Winnebah respectively, and to Dr. P. S. Selwyn-Clarke for making it possible for us to visit, in search of material, a number of the localities in the neighbourhood of Accra.

COLLECTION

ADULTS. A few specimens were taken in bungalows at night, either on the wall in the vicinity of a lamp or on the hand when writing. We also received from Dr. Storey several large collections of *Culicoides grahami*, Aust., taken in the act of biting, at Koforidua and at villages in the neighbourhood of that town. The majority of the adults collected were, however, obtained in the Accra laboratory.

It was our custom to collect small diptera in the laboratory every evening between the hours of 5-30 and 6-30, that is, during the

brief tropical twilight. They were secured in the following manner. At 5 p.m. the windows of the laboratory were closed. About half an hour later, midges began to appear on the inside of the window-panes and were collected until nightfall by placing a small tube containing potassium cyanide or wool sprinkled with chloroform over them as they rested on the glass, closing the tube by inserting a visiting card between it and the window-pane, and then lifting it away with the card still in position. If the windows of the laboratory happened to be closed during the day-time a few midges were occasionally seen on them, but it was not until nightfall that any considerable number appeared. So far as could be judged, they were endeavouring to escape, for they could sometimes be seen flying towards the light, and it is probable that they had taken refuge in the building during the day-time or in the early hours of the morning.

The collections thus made contained a large number of interesting forms and many different kinds of flies, amongst them numerous examples of *Phlebotomus*, *Culicoides* and *Forcipomyia*, both males and females. Specimens of *Culicoides* could be recognised with fair accuracy on the window-panes by their small size, dark-grey colour, and somewhat humped-back appearance. They seemed to have but a feeble foothold on the glass, and, unlike *Phlebotomus*, did not jump about from place to place but remained stationary, generally near to the wooden framework of the window, or crawled slowly along the glass. The males, by reason of their plumose antennae, were slightly more conspicuous than the females.

In these evening hunts for insects we were frequently joined by predaceous insects and small lizards. The lizards were not observed to attack midges, but on one occasion a small Empid pounced upon and carried off a specimen of *Culicoides* that we were endeavouring to capture. The same insect, which, through the courtesy of the Imperial Bureau of Entomology, has been identified as a species of *Elaphropeza* by Mr. J. E. Collin, preyed also upon *Forcipomyia ingrani*, Cart., and other, at present unidentified, species of this genus. Specimens of *Phlebotomus* (probably *P. minutus* var. *africanus*) were repeatedly seen, engorged with blood, on the heads, limbs and bodies of the lizards; they were most tenacious of their hold, refusing to let go even when the lizards were moving actively.

The lizards appeared to be quite indifferent to the attacks of these insects.

During the five months of the investigation with which we are at present concerned, over a thousand specimens of *Culicoides*, *Forcipomyia*, etc., were collected in this way, including representatives of at least twenty different species. This is of considerable interest in view of the fact that midges are not especially troublesome at Accra. A number of the specimens on examination appeared to be engorged with blood. It is noteworthy that we were never consciously bitten by any insects other than mosquitoes whilst making these collections in the laboratory, but it should perhaps be mentioned, although it may have no significance, that the one of us who made most of the collections suffered from nine separate short attacks (one to seven days) of fever during the period, and that on none of these occasions were malaria parasites found in his blood.

LARVAE AND PUPAE. Larvae and pupae were obtained by collecting samples of the water, mud, or vegetable matter from small pools, rot-holes in trees, old canoes, crab-holes, the rotting roots of banana plants, etc. The pupae of aquatic forms, such as *Culicoides*, could sometimes be seen floating on the surface of the water at the edge of pools, in dug-out canoes, etc., and could be collected by means of a dipper; occasionally larvae also could be seen, but their small size and their habit of burying themselves when disturbed made this a very difficult matter. As a rule, it was not possible to determine in the field if larvae and pupae were present, therefore we soon adopted the plan of collecting samples of any materials we thought likely to harbour the insects and transporting them to the laboratory for examination at leisure. In this way a large number of samples was examined from a great variety of situations.

The materials from which we obtained larvae and pupae may be considered, so far as the methods of collection are concerned, as belonging to one of two types: samples of moist débris, and fluid samples.

The samples of moist débris included such materials as the mud from the edges of pools, the vegetable matter collected at the bottom of rot-holes in trees, the rotten wood of canoes, and the decomposing matter from the bases of banana plants. Such materials were

carried to the laboratory in cigarette tins or small jars, and were then turned out into larger glass jars and covered by glass plates. Water was added to these samples in such a way that only about one-half of the material was submerged, so that the conditions might be suitable for both aquatic and terrestrial species.

The fluid samples, which were obtained from pools and puddles, rivers, crab-holes, bamboo-stumps, rot-holes in trees, etc., included some of the mud or other débris forming the natural bottom, and were usually collected in glass jars which only required to be securely covered on their arrival at the laboratory. If, however, they had to be transported a long distance by train, motor car, or bicycle, involving continual joltings, we found it preferable to drain off most of the water by pouring the sample on to a pad of filter paper or vegetable matter contained in a tin, the bottom of which had been pierced in one or two places. In this connexion it may be mentioned that a sample of the contents of a rot-hole in a tree containing mosquito larvae, which was sent to us through the post by a modification of Legendre's (1916) method of transportation, which we have found of considerable value, was found on arrival to contain also living larvae of *Culicoides*.

All the samples collected were kept at least a week before being discarded. This was done because a day or two often elapsed before larvae were discovered in them, and in order that any buried pupae present might have a chance of hatching.

SEGREGATION

From many samples there emerged several different species of midges. The small size of these insects, and the frequent slight specific variation in the larvae and pupae generally rendered them indistinguishable in life, and, therefore, it was necessary, in order correctly to correlate the immature and adult stages of a species, to obtain the larval and the pupal pelts of single individuals. The neglect of this precaution would inevitably result in incorrect associations.

For this purpose we isolated single larvae or pupae in small glass tubes, and in several cases succeeded in rearing the adults

and in recovering the pelts. It will be readily understood, however, that this was not easy, and that in many cases we were unsuccessful. In order to be able to find such a minute object as the crumpled and collapsed larval pelt, it was necessary to reduce the amount of the medium to a minimum, so that there was danger of starving the larva or rendering the conditions otherwise unsuitable for its existence. It was also necessary, of course, to examine very carefully the débris placed in the tubes to see that it contained no other small larvae, old pelts, or predaceous insects. In the case of *Culicoides*, the larvae and pupae of which are aquatic and transform at the surface, the pelts were comparatively easy to find, as they generally remained floating; in the case of *Dasyhelea* it was more difficult, as they were left embedded in the solid medium and had to be sought by teasing out the materials under a dissecting microscope.

EXAMINATION

ADULTS. These small insects shrink greatly and unequally during the process of drying, so that morphological characters are obscured and little reliance can be placed on measurements. Such specimens, however, are all that are usually available. The individual variations in size are moreover considerable, and any measurements that can be given are therefore only approximate. The most reliable and satisfactory measurements, because they are least subject to the effects of shrinkage, are those of the wing; in freshly killed midges the ratio of the length of the wing to the length of the body, excluding the head, was found to be approximately as 1 is to 1.2.

Those measurements which we have selected, the length of the body and the length and breadth of the wing, are, unless otherwise indicated, made from mounted specimens. We have recorded the length of the body from the anterior margin of the thorax to the posterior end of the abdomen. The head is excluded, because one of the most pronounced and variable effects of drying is the retraction of the head under the thorax: in some specimens it is almost completely hidden, in others it is only slightly retracted. Measurements of specimens after immersion in pure carbolic acid are very

close to those of the living insect. This medium has the effect of clearing and distending the dried midges, and if used carefully does not over-distend them. It is, therefore, a most convenient fluid in which to mount specimens for the examination of morphological characters.

Another effect of drying is to obliterate delicate markings and to obscure shades of colouring. Dried midges are with few exceptions brown; in life they are more often a dark olive-green colour. Here again, however, we have thought it best to describe the conditions seen in dried specimens, contenting ourselves with a brief reference to the appearances in fresh specimens only when the differences were notable. It should be understood, however, that in the living insect the markings are almost invariably brighter and clearer, and that many a thorax or abdomen, which in dried materials appears uniformly brown, may have been originally beautifully but delicately patterned.

The drawings of the wings were made from dried specimens. After immersion in pure carbolic acid, or when mounted in balsam, the pale markings appear to be larger and more diffuse. In examining the thoracic ornamentation or the wing markings, it is necessary to have unshrunk specimens and to view them from all points in order to obtain the effect of light falling on them at different angles. It is possible to form an entirely erroneous conception of the pattern if only shrunken specimens are available, or if they are viewed with light falling on them from one direction only. With a hand lens ($\times 16$) or a low power of a binocular microscope the patterns are appreciated best; under higher powers, such as those of an ordinary microscope, the patterns tend to break down. A low power of a binocular microscope was used for the descriptive work in the succeeding papers.

It may be mentioned here that, apart from antennal and genital characters, there are not great differences between the sexes in these midges. The male in most respects is similar to the female, but smaller, and of slighter build. In general the dark markings in the male tend to be more diffuse and conspicuous than in the female.

PUPAE. The best materials for examination are specimens which have recently pupated or pelts. The pupal case is sufficiently

rigid to retain its form after the emergence of the adult insect. Such cases are more suitable for study than the pupae themselves, as the armature of spines and tubercles is not obscured by the hairs and organs of the enclosed insect. In older pupae the enclosed insect can be seen clearly, and after treatment with clove oil, pure carbolic acid, etc., can be examined in detail. The most convenient medium for the examination of the external structure is pure carbolic acid in which, when mounted on a hollow slide, the pupa or its pelt can be rolled from side to side and viewed from every aspect.

LARVAE. The larvae should be fixed by dropping them into hot fluid (alcohol, formalin, or water) so as to prevent undue shrinkage and the retraction of the anal gills. For examination pure carbolic acid is again a very convenient medium; it clears the head thoroughly but renders the body rather too transparent. This drawback, however, can be easily corrected by diluting the medium with alcohol.

Larval pelts are not very suitable for detailed examination, owing to the fact that the body segments are more or less telescoped and the head is split on each side dorsally.

THE VALUE OF PURE CARBOLIC ACID IN THE STUDY OF SMALL INSECTS. In the study of these small midges we have found pure carbolic acid a reagent of the highest value. Reference has already been made to the fact that in this medium the dried insects are distended so that they reassume their natural dimensions. This action is of value, not only because it enables accurate measurements to be made of the various parts, but also because the most minute structures, such as sculpturing on the antennae and sensory hairs on the palpi, can be clearly seen and examined with high powers of the microscope.

The advantages which we claim for this medium are the following:—

1. It rapidly clears specimens preserved dry, in alcohol, or in watery solutions such as formalin, without shrinkage or distortion.
2. It restores the specimens to their natural forms, and in doing so re-expands such organs as the palpi, the antennae, claspers, etc.
3. It does not denude the specimens.
4. In it, on a hollow slide, the specimens (adults, pupae, or

larvae) can be 'rolled' from side to side, thus enabling any particular object to be examined from every aspect. This is a very important advantage in studying the genitalia, the spines on the antennae, the tubercles of pupae, etc.

5. At the end of each examination the specimen can be put away in a tube of alcohol or formalin, and subsequently taken out for re-examination as often as required; or it can be permanently mounted in Canada balsam.

Except when the structures are very dark and very highly chitinised, pure carbolic is, in our opinion, preferable to caustic potash as a clearing agent. The latter reagent takes longer to act, softens the tissues so that they are unnaturally flattened even by the weight of a cover slip, and, owing to the subsequent manipulations attending washing and dehydration, is apt to denude the hairs and to distort the more delicate structures to a misleading extent.

BIONOMICS

ADULTS. The midges are phototropic. When reared in glass jars in the laboratory they are always found resting or crawling about upon the side nearest the strongest light; reversing the position of the jars causes them to change their positions. If they have been reared in a small glass tube they are easily induced to leave it and ascend into another tube by the simple expedient of inverting the one tube over the other and pointing the bottom of the upper one towards a strong light.

In the case of some species at any rate, males and females appear to hatch at different times. In one instance, from débris procured from a rotting tree, thirty-two males of a species of *Culicoides* (*C. clarkei*, sp. n.) emerged in succession before a single female appeared, then a series of a dozen females hatched, followed by two more males. Of another species (*C. eriodendroni*, sp. n.) forty females were reared but not a single male.

A newly hatched *Culicoides* is capable of walking about as soon as it leaves the pupal case, and of flying within ten minutes of its emergence. In the case of *Dasyhelea*, if the pupae have become dislodged from their natural positions in rotten wood or other

vegetable matter, and are floating in water, the adults seldom succeed in hatching completely.

As has already been said, most of our specimens were obtained in the evening on the windows of the laboratory under circumstances which suggested that they were attempting to escape from the building. At the same time, when we were collecting large numbers of specimens in the laboratory, very few were to be found in our bungalows which were not far distant, and those that were found appeared to have been attracted by lights. This difference may have been due to the fact that whereas the laboratory was closed at night our bungalows were more or less open at all times.

During the five months of this investigation midges belonging to the genera *Culicoides* and *Forcipomyia* were always more or less numerous in the laboratory in the evening. Some of the species were abundant throughout, some appeared to show a seasonal variation, and others were taken only on rare occasions. At the end of the description of each species we have recorded the months during which it was collected, and have noted any peculiarities regarding its prevalence. It may be stated here, however, that in December, 1919, when the investigation was started, midges were fairly common, and some six species of *Culicoides* and three of *Forcipomyia* were secured. Throughout January all but one of the same species were taken in larger numbers, and in addition several new species were collected. The total number of specimens captured in January was the largest of any month during the period under consideration, fifteen or twenty specimens of *Culicoides* alone being taken each evening, and on one particular occasion no less than seventy. In February, the height of the dry season, much the same species were collected, but in greatly reduced numbers; indeed several may be said to have practically disappeared, as only single specimens were obtained. In March the commoner species again increased, and several new species were secured, so that in this month the largest number of different species was collected. In April collection was carried out less systematically, and this may have accounted for the fact that certain of the rarer species were not taken, although all the commoner ones were abundant. In January, therefore, the largest number of specimens was collected; in March the greatest number of different species. *Forcipomyia ingrami* was

the commonest species of midge upon the laboratory windows during the period, a fact that is readily explained since it was being artificially reared at the time; next to it in frequency of occurrence was *C. schultzei* (End.). It may be mentioned, although it does not properly concern us here, that during May and June, with the onset of the rainy season, the number of *Culicoides* fell off greatly and the number of *Forcipomyia* increased.

EGGS. Except in the case of *F. ingrami*, no attempts were made to obtain oviposition with any of the midges captured. On one occasion, however, a female of *F. castanea*, Wlk., was found which had a few eggs attached to the extremity of the abdomen. These were dark, elongate oval, somewhat pointed at the ends and surrounded with what appeared to be a delicate gelatinous capsule; the average measurements of these were, length 240μ , greatest breadth 80μ . The abdomens of several captured females of various species of *Culicoides* and *Dasyhelea* were seen, on examination in carbolio, to be filled with eggs. These, in both genera, are relatively large and appear to be very long and narrow and slightly curved with bluntly rounded ends (sausage-shaped); the average dimensions of three such eggs of *C. grahami*, Aust., were, length 303μ , greatest breadth 45μ .

LARVAE AND PUPAE. It will be convenient to record the few bionomical observations we have made on species belonging to the genera *Culicoides* and *Dasyhelea* separately, as these are the only genera of which we have hitherto succeeded in procuring and identifying a sufficient number in their early stages. Any observations we have made on other species will be included in the text of the systematic descriptions. A full account of the early stages of *Forcipomyia ingrami* has already been given by one of us (Carter, 1919); this midge was reared frequently in the laboratory and its larvae were found twice during this investigation in material taken from the base of a banana stump at Nsawam, and from a rot-hole in a cotton tree at Oblogo.

CULICOIDES. The only true members of this genus of which the larval and pupal stages have been described in any detail, appear to be *C. kiefferi*, Patt. (Patton, 1913) and *C. pulicaris*, L. (Goetghebuer, 1919).^{*} We were successful in rearing nine different

^{*} In a previous paper (1914) Goetghebuer gives an excellent account of the early stages of *Dasybelea versicolor*, Wtz., which he places in *Culicoides*.

species of *Culicoides* from materials collected in the manner described. They were found in rot-holes in trees (Plates V and VI, figs. 4, 6 and 8), such as the flamboyant (*Poinciana regia*), the silk-cotton (*Eriodendron anfractuosum*), the cashew (*Anacardium occidentale*), the mango (*Mangifera* sp.), a species of *Cynometra*, probably *C. megalophylla*, and one or two other species not determined; in the partly decomposed and water-laden roots or bases of banana stumps (Plate V, fig. 5); in canoes tied to the banks of rivers (Plate VI, fig. 7); in a backwater of a river which was used as a washing-place; and in pools and puddles such as are frequently found in the vicinity of stand-pipes (Plate IV, figs. 2 and 3). In the systematic descriptions of species, which will be published later, notes will be given of the situations in which early stages were collected. Larvae which appeared to be those of some species of *Culicoides* were also obtained from a clump of bamboos by a river, from crab-holes at the edge of a lagoon, and from the margins of a large pool, but were not reared through to the adult stage.

With one exception, all the species of *Culicoides* reared had spotted wings. The single clear-winged species was bred from materials obtained from a rot-hole in a cotton tree and from the base of a banana plant, and it is noteworthy that this was the only *Culicoides* found in the latter situation which was, however, a favourite breeding-place of several species of *Dasyhelea*.

It is perhaps worth recording that, although twelve species of *Culicoides* were captured upon the laboratory windows between the beginning of December and the end of April, with the exception of *C. accraensis*, sp. n., of which the larvae and pupae were found floating in water accumulated in a rot-hole in a flamboyant tree close to the laboratory, the early stages of none of the others were found in any collection of water within a radius of 150 yards of the laboratory, despite diligent search for them upon many occasions.

Small samples of materials occasionally produced very large numbers of midges; they also sometimes continued to yield larvae for a long time. In one instance some material taken from a rot-hole in a flamboyant tree on the 10th December continued to produce larvae (*C. accraensis*) in gradually diminishing numbers until the 20th March.

From the samples of material collected there emerged often more than one species of midge, and from some, such as that taken from a rot-hole in a cotton tree at Nsawam, as many as six different species of *Culicoides* were reared. It was not possible in every case to isolate the larvae and pupae, but we were fortunate enough to procure the larvae of five and the pupae of seven species.

The larvae are slender, eel-like creatures, about 3 mm. long. All those that we collected were somewhat similar in their appearance and, so far as they were observed, in their habits also; but the species for which we propose the name *C. accraensis* was the one we were able to procure most abundantly and to study most closely at Accra, and, therefore, the observations recorded below refer particularly to it—points of difference with regard to other species only being noted.

The colour of the body of the larvae is dull white, and it usually remains unaltered until pupation; the head is yellowish-brown. In the cases, however, of three larvae which came under our observation, two collected from a crab-hole at Christiansborg, a suburb of Accra, and one from a bamboo at Oblogo, the colour gradually darkened to a mahogany brown. These larvae, unfortunately, died, and it is not known to what species they belonged, or whether indeed the dark colour may not have been due to some pathological condition.

The larvae normally develop in water, but appear to be capable of surviving for a time in moist situations where no free water is present. That they can survive for at least six days without actual immersion in water is indicated by the two following experiments.

1. A large sample of débris scraped from a rot-hole in a flamboyant tree was divided into two equal portions and each portion placed in a glass jar and covered. To one portion water was added, so that the solid matter was almost completely submerged: *Culicoides* larvae (*C. accraensis*) were observed in this jar the next day, pupae on the fifth day, and adult insects on the ninth day. The other portion was not treated in any way: no larvae, pupae, or adult midges were seen in this jar during an observation period of ten days.

2. Débris scraped from a rot-hole in another flamboyant tree was similarly divided into two equal portions. To one portion

water was added: larvae of *C. accraensis* were seen in this jar two days later. The other portion was left untouched; six days later, as nothing had appeared in this jar, water was added, and two days after this well-grown larvae of *C. accraensis* were observed.

The movements of the larvae in water are active, and are effected by means of a vibratile motion of the whole body, which has been compared with that of a spirochaete (Patton, 1913). The movements are intermittent. Notwithstanding these convulsive movements, progression, especially when descending from the surface is not very rapid, and is slower than that of a mosquito larva of the same size.

If when swimming at the surface the larvae are disturbed, they work their way downward and burrow, head first and with extraordinary rapidity, into the débris at the bottom and disappear from view completely. If the jar be then left undisturbed for an hour or so they reappear, but do not always leave the débris entirely. They seem, indeed, to spend the greater part of their existence buried in the fine mud at the bottom with only their heads and the first three or four body-segments projecting, an attitude in which they resemble tiny protruding roots of plants. When thus situated, any movement, or even the slightest vibration, causes them immediately to retract completely.

Larvae buried in the débris at the bottom of a vessel may frequently be induced to come to the surface by replacing the supernatant water by fresh water. The larvae appear to be susceptible to light. If kept in rectangular glass jars they will usually be found, when at the surface, at that side or angle of the jar directed towards the strongest light. In this respect they behave in a manner similar to the larvae and pupae of certain mosquitoes, such as *Stegomyia fasciata*, Fabr.

The larvae appear to survive transportation well, and may be carried either in the natural watery medium, in moist débris, or stranded on moss or filter-paper. On one occasion a sample containing larvae of *Culicoides* was received safely through the post.

The duration of the larval stage was not determined in any of our species, but it is probably largely influenced by the food supply, and may certainly extend over several weeks.

When about to pupate the larvae frequent the surface, usually

hugging the side of the jar next to the light. They often may be seen twining themselves into loops and S-shaped curves about small floating particles. It is possible that these raft-particles assist in the separation of the pupae from the larval pelts, because the latter are often found attached to them; but this fact may be due to surface tension, and pupation can occur quite readily in the absence of any such floating particles. At this stage the three anterior segments of the body are much enlarged and the eyes are situated far back in the head.

Pupation takes place at the surface. The pupa emerges through the anterior part of the body of the larva, leaving a pelt in which little can be distinguished but the head and the long hairs at the posterior end of the last body segment. The larval pelt may often be found floating beside the pupa, from which, however, it is entirely separated. It is a very small object, and is not suitable for detailed study because the abdominal segments are telescoped and the posterior parts of the head is split on each side of the median dorsal plate (clypeus), from its posterior margin to a point a little behind the anterior end.

A detailed description of the pupae will be given later. Those of males are smaller than those of females. When newly emerged from the larval skin the pupae are almost white but speedily change to a golden-brown colour which darkens before the adult insects emerge, becoming in some species almost black.

The pupae are aquatic, and float in a vertical position with the body extended and the trumpets in contact with the surface. They are sluggish and exhibit only slight translatory movements; they are, however, more active than the pupae of *Forcipomyia ingrami*, and if stranded on the sides of a glass vessel by tilting, are quite capable of wriggling back to the water over a distance of at least one inch. Such movements as they do exert are effected by antero-posterior and lateral motions of the abdomen. None of the pupae observed by us employed the processes at the posterior end of the abdomen as hooks to anchor themselves as, according to Patton (1913), do those of *C. kiefferi*.

The pupae are very easily killed. They do not appear to be able to survive submergence for any length of time, and consequently do not well withstand transportation in vessels containing

water. They will live, however, and the adult insects will hatch from them readily enough if they are stranded upon a solid surface, provided that they are kept moist and are laid upon their ventral surface so that air can enter their respiratory trumpets.

The duration of the pupal stage is from three to five days. Warning is given of the approaching emergence of the adults by an apparent darkening of the pupae, the change of colour being due to the darkening of the enclosed insect. When the adult insect emerges, the pupal case splits down the middle of the dorsum of the cephalo-thorax from the level of the bases of the respiratory trumpets to a point a little in front of the posterior margin; the antero-median portion of the cephalo-thorax is also split on its lateral and posterior sides and raised like a lid or operculum. After the escape of the adult insect, the pupal pelt retains its shape, and remains, full of air, floating on the surface. It is very suitable for examination on account of its translucency and because the parts are but little distorted.

DASYHELEA. We were successful in rearing ten different species of *Dasyhelea* from materials collected, in the manner described, from rot-holes in trees, such as the flamboyant, the silk-cotton, the mango, a species of *Cynometra* probably *C. megalophylla*, and another tree not specifically identified, from the partly decomposed roots or bases of banana stumps, and from the rotted wood at the sides and ends of canoes (c.f. Plates V and VI). The majority of the specimens of *Dasyhelea* obtained were, indeed, reared in this way, only a few being collected on the windows in the laboratory or on the walls in a bungalow at Accra.

As in the case of *Culicoides*, there often hatched from small samples of materials large numbers of specimens belonging to several different species. For example, from one portion of rotted banana fibre, which weighed about a pound and a half, more than a hundred specimens emerged in the course of the five weeks it was kept under observation. Five different species of *Dasyhelea* were obtained from this small sample.

Owing to the occurrence of more than one species in many samples, and because at first we did not know the conditions necessary for the rearing of isolated individuals, we were unable to secure the early stages of all the species bred. We did, however,

succeed in procuring the larvae of four or five and the pupae of seven species.

The larvae are similar in appearance to those of *Culicoides*, and have yellowish-brown heads and creamy-white bodies. The cuticle covering the body is very thin and almost transparent, so that the internal organs can be seen through it. When living in dark coloured débris the larvae may, therefore, appear to be brownish, owing to the presence of ingested matter in the intestine.

The larvae live buried in moist fibrous débris, such as rotten wood or the decaying stumps of banana plants. They were never seen on the surface of such materials, but were discovered only by teasing out with needles small portions of the samples. When isolated in small tubes containing a little moist fibrous matter the larvae rapidly buried themselves and were not again seen at the surface.

Although requiring moisture for their development, the larvae are not truly aquatic, and are unable to survive submersion in water. Their natural habitat appears to be the interstices between the fibres of vegetable matter, preferably when rotting, where there is a certain amount of moisture. If the materials in which they are living are flooded with water the larvae die, but if only sufficient water is added to keep the mass moist they thrive well in glass jars, pupate, and subsequently attain the adult form.

The movements of the larvae are relatively sluggish. When placed in water they do not swim with active vibratile movements in the manner of the larvae of *Culicoides*, but crawl about laboriously at the bottom. They crawl rather more actively over solid materials such as banana fibre, and rapidly bury themselves in them, and they are capable of climbing up the sides of a glass jar for a distance of a few inches. The manner of ascent is either a slow crawling or by a bending of the body laterally into a loop, so that the posterior end is brought close to the head, followed by a straightening of the body which forces the head end upward.

The larvae survive transportation well and may be carried in their natural medium, provided that it is not completely water-logged, in jars or tins, or any other convenient receptacle. The duration of the larval stage was not determined in any of the species we collected, but it appeared to be long (several weeks) in some

cases, and is probably largely dependent on the food supply and the temperature.

Owing to the fact that the larvae live buried out of sight in moist fibrous materials, their behaviour under natural conditions could not be observed. When about to pupate, however, they appear to come close to the surface. The larval pelt may often be found lying close to the posterior end of the pupa, but separated from it. It is a very small and inconspicuous object, because the body segments are telescoped. The pupa appears to emerge from the larval skin in the same manner as in *Culicoides*.

The pupae resemble those of *Culicoides*, but may sometimes be distinguished by the shape of the respiratory trumpets. A detailed description of the morphological characters will be given later. They are not truly aquatic, and are not naturally found floating in water. Pupation takes place close to the surface of the material in which the larvae develop; the pupae remain embedded in it with their bodies outstretched, the respiratory trumpets and the dorsal portion of the cephalo-thorax only protruding. They are, therefore, invisible to the naked eye, and even after the adult insects have emerged the pelts are difficult to find, because they remain partially embedded in the fibrous matter.

The pupae of *Dasyhelea*, if removed from their natural positions and laid on damp filter-paper, are more inert than those of *Culicoides*; they make no attempt to alter their position unless they have been placed on their backs, when they try to turn over so as to bring their dorsal side uppermost. When placed in water they float at the surface in the same manner as *Culicoides* pupae, but do not long survive. A large number of pupae of two species of *Dasyhelea* were found on several occasions in water collected at the bottom of canoes tied to the bank of the river Densu at Oblogo: the majority of them were already dead or died during the week they were kept under observation, and the few adults which did emerge were found dead on the surface of the water or incompletely detached from the pupal cases. Somewhat greater success followed when the pupae were removed from the water and laid on damp filter-paper, but even then the large number of adults which failed to emerge completely suggested that the conditions were unnatural. It was found later that the rotting wood at the ends and sides of

these canoes was a favourite breeding place of several species of *Dasyhelea*, and it is probable, therefore, that the pupae found in the water in the canoes had been washed by rain from these situations.

The duration of the pupal stage is three or four days. As in the case of *Culicoides*, warning is given of the approaching emergence by an apparent darkening of the pupae. After the escape of the adult the pupal case may be found still partially embedded in the natural fibrous medium. It retains its shape, and is filled with air and floats to the surface if liberated by teasing the materials under water. When the adult insect emerges the pupal pelt is split in the same manner as that of *Culicoides*.

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EXPLANATION OF PLATE IV

Breeding-places of *Culicoides schultzei* (End.).

Fig. 1 Small pool by railway line.

Figs. 2 and 3. Puddles near stand-pipe.



FIG. 1

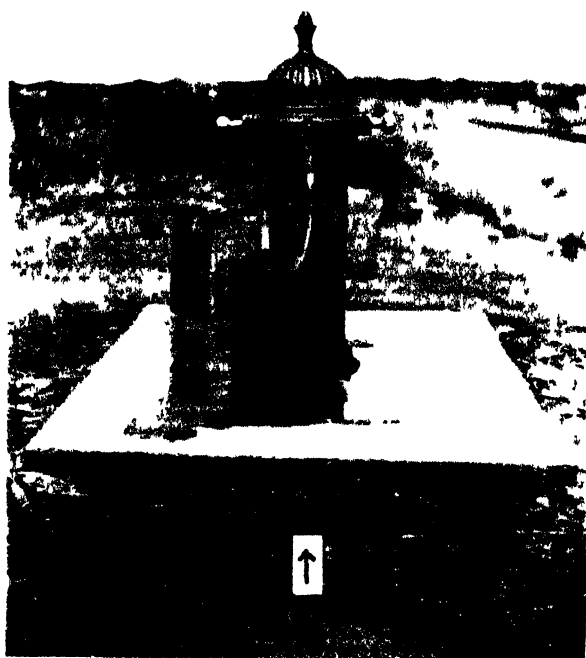


FIG. 2



EXPLANATION OF PLATE V

- Fig. 4. Rot-hole in fork of flamboyant tree (*Poinciana regia*); breeding-place of *Culicoides accraensis*, sp. n.
- Fig. 5. Stumps of banana plants; breeding-places of *Culicoides inornatipennis*, sp. n., *Dasyhelea* spp. (three species) and *Forcipomyia ingrami* (Cart.).
- Fig. 6. Rot-hole in a species of *Cynometra*; breeding-place of *Culicoides accraensis*, sp. n., *C. clarkei*, sp. n., and *Dasyhelea* spp. (two species).



Fig. 1



Fig. 2



Fig. 3

EXPLANATION OF PLATE VI

- Fig. 7. Partially waterlogged canoes; breeding-places of *Culicoides schultzei* (End.), *C. similis*, sp. n., and *Dasyhelea* spp. (three species).
- Fig. 8. Rot-hole in the stump of a silk-cotton tree (*Eriodendron anfructuosum*); breeding-place of *Culicoides accraensis*, sp. n., *C. clarkei*, sp. n., *C. eriodendroni*, sp. n., *C. inornatipennis*, sp. n., and *C. punctithorax*, sp. n.



Fig. 7



Fig. 8

OBSERVATIONS ON THE
CERATOPOGONINE MIDGES OF THE
GOLD COAST WITH DESCRIPTIONS OF
NEW SPECIES

PART II.

BY

HENRY F. CARTER

A. INGRAM

AND

J. W. S. MACFIE

(Received for publication 14 September, 1920)

PLATES VII AND VIII

SYSTEMATIC ACCOUNT

Since the literature relating to the classification of the *Ceratopogoninae* reveals some confusion in the conceptions of certain genera, and in view of our imperfect knowledge of the biology of these little flies, it has been considered advisable to preface some of the genera dealt with by an account of the external morphology of the adults and immature stages. These accounts are drawn up from the material contained in our collections, and conveniently summarise the characters common to all the species we have examined. The subsequent specific descriptions are thus made as brief as possible.

The types and co-types of the new species described herein have been deposited in the museum of the Liverpool School of Tropical Medicine.

Genus *CULICOIDES*, Latr.

Ceratopogon, Meig. (pro parte). Illiger's Mag. Ins. Vol. II, 1803.

Culicoides, Latr. Gen. Crust. et Ins. Vol. IV, 1809.

Haematomyidium, Goeldi. Mem. Mus. Paraense, 1905.

? *Oecacta*, Poey. Mem. Hist. Nat. Cuba. Vol. I, 1851.

EXTERNAL MORPHOLOGY

ADULTS. *Head.* Eyes bare; in the female usually separated dorsally by a narrow, parallel-sided, strip of integument which is bounded at the vertex by an internal transverse chitinous band, immediately below which is a single strong hair; in the male usually more widely separate than in the female, with a Y-shaped chitinous thickening extending from the vertex to the middle of the frons (fig. 1). Vertex and occiput, in both sexes, with numerous,

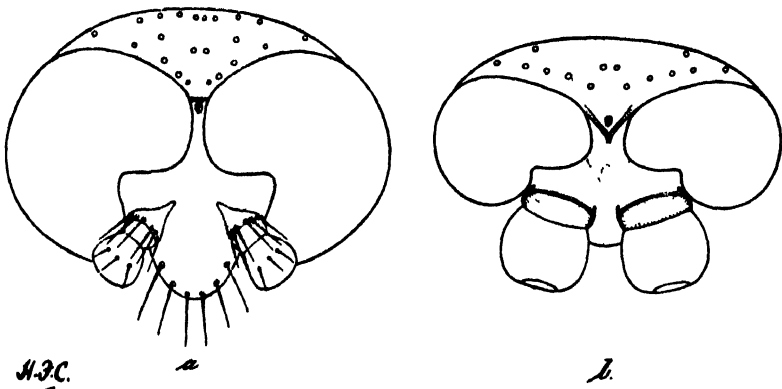


FIG. 1. *C. distinctipennis*, Aust. a—head of female; b—head of male. (× 140.)

stout, forwardly projecting hairs. Clypeus moderately pronounced, hairy.

Mouth-parts. Proboscis about as long as the head in the female, shorter in the male. The component organs of the female proboscis are shown in fig. 2. The labium is fleshy and bears a few relatively long, and several short hairs. The remaining organs are strongly chitinised and serrated distally. The labrum is armed with three terminal recurved teeth and four

teeth on each side. The broadly rounded distal margin of the hypopharynx is divided into several minute, closely apposed teeth. The mandibles and maxillae are each armed with several (fourteen to sixteen) strong teeth, those of the maxillae being relatively large and widely separated. In the male the labium is similar to that of the female but is rather narrower; the remaining organs are shorter, more delicate and less powerfully armed. The labrum, although provided with small sub-apical recurved teeth, is devoid of lateral teeth, and the extreme apex is drawn out into a membranous fringe. The hypopharynx is well-developed but is less

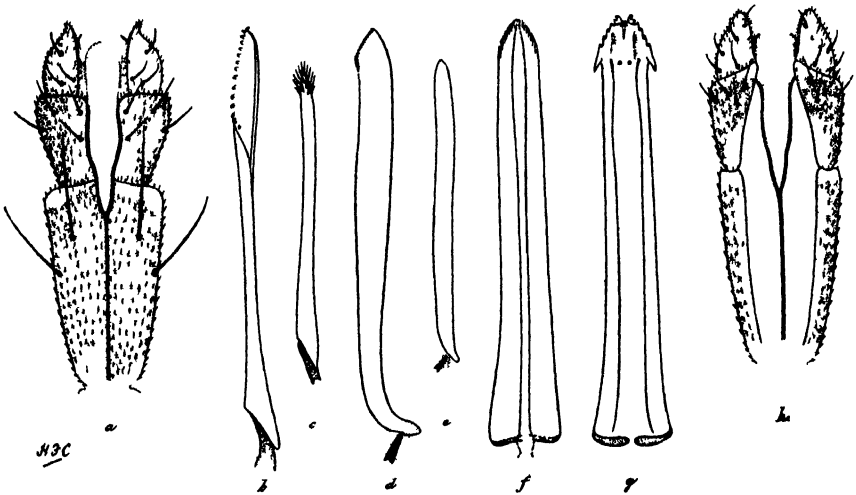


FIG 2. Mouth-parts of *C. austeni*, sp. n. a—labium, ventral view (♀), b—maxilla (♀), c—maxilla (♂), d—mandible (♀), e—mandible (♂), f—hypopharynx (♀), g—labrum (♀), h—labium, dorsal view (♀) (All $\times 270$ circa)

heavily chitinated than in the female, and distally narrows to a finely rounded apex bearing two or three minute hairs. The mandibles (fig. 2 e) may or may not be serrated, but the teeth, when present, are reduced in size and number. In the males of *C. schultzei* (End.) and *C. distinctipennis*, Aust., each mandible bears four teeth. The maxillae (fig. 2 c) differ considerably from those of the female; they are rather sharply attenuated distally, and the extremity is covered with minute hairs. The palpi (fig. 3), in both sexes, are composed of five segments, bearing moderately long and short hairs, the first, fourth and fifth segments short, the second and third long, usually

sub-equal, each about twice the length of any of the other three; third segment expanded near the middle or anteriorly and containing a deep pit or sensory cup, opening on the inner aspect, in which are a number of minute modified drum-stick-like hairs. Palpi of the male smaller than those of the female.

Antennae (fig. 4). Antenna of the female composed of fifteen segments, pilose; the torus (second segment) dark brown, the flagellum (segments three to fifteen) paler brown with the last two or three segments slightly darker. All the segments are sharply

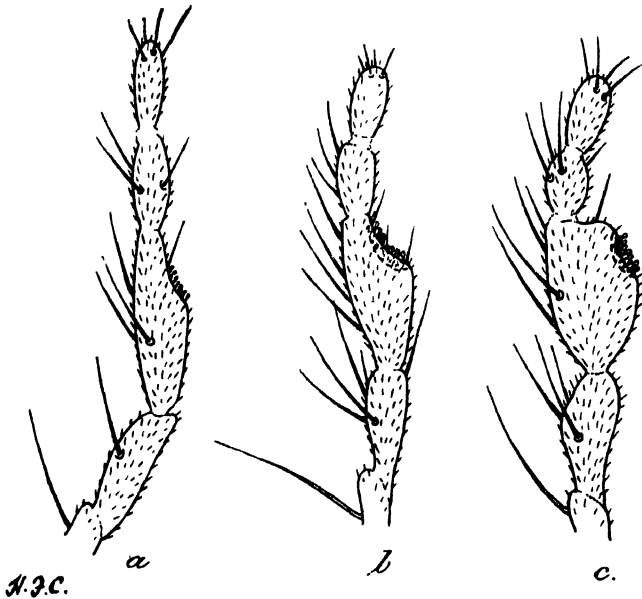


FIG. 3. Palpi of females of: a—*C. austeni*, sp.n.; b—*C. distinctipennis*, Aust.; c—*C. accraensis*, sp. n. ($\times 330$.)

separated one from another. First segment small with a few relatively stout hairs arising anteriorly and reaching to about the middle of the torus; the latter sub-spherical with five or six rather short hairs arising near the anterior margin. Third segment ovoid and stalked posteriorly, larger than the fourth. Segments four to ten sub-spherical, ovoid or sub-cylindrical, becoming progressively longer towards the tenth; segments eleven to fifteen elongate, sub-cylindrical, each from three to four times as long as the greatest breadth. The terminal segment is tapering and ends in a bluntly

rounded apex. Hairs on the basal segments short, about twice the length of the segments, arranged in whorls; each segment bears a whorl of five or six hairs, and short slightly curved dorsal spines arising anteriorly. On the third segment are nine or ten hairs,

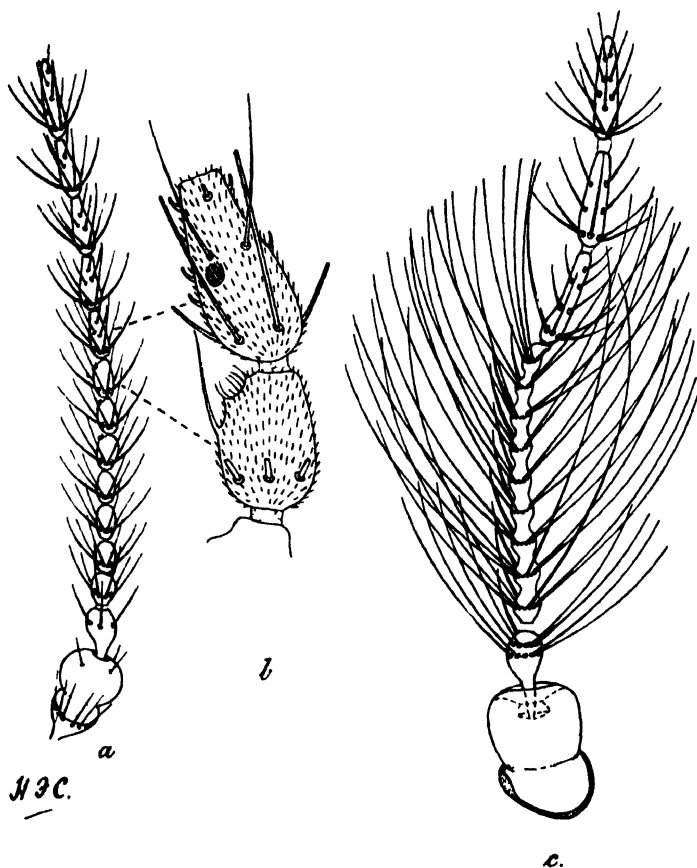


FIG. 4. *a*—*C. schultzei* (End.), antenna of female; *b*—tenth and eleventh segments more highly magnified; *c*—*C. distinctipennis*, Aust., antenna of male (comparatively few of the plume hairs are shown). (*a* and *c* $\times 140$, *b* $\times 550$.)

sometimes arranged in two incomplete whorls. Hairs on the five distal segments usually rather shorter than the segments, those arising posteriorly forming whorls of six to eight hairs, the rest scattered; several very small curved spines occur on each of the last five segments. Minute pits bordered with hairs are present on all

the flagellum segments; three or less are usually present on each segment, but the number and distribution varies.

Antenna of the male composed of fifteen segments, slightly longer than that of the female, plumose, the hairs adpressed and not standing out from the shaft when the insect is at rest or dead. First segment broad, chiefly membranous, without hairs. Torus very large, sub-spherical. Basal portion of the third segment elongate; the apical portion ovoid, with two whorls of about ten hairs each, separated from the fourth segment by a wide membranous interspace. Fourth to twelfth segments short, ovoid, broadly united and not very distinctly separated one from another; last three segments elongate, cylindrical, and sharply separated, sub-equal in

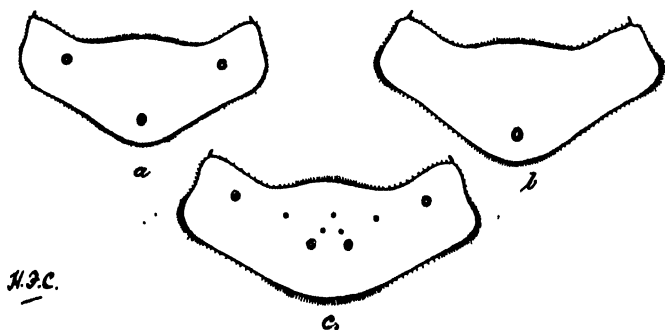


FIG. 5. *C. grabams*, Aust. *a*—scutellum of female; *b*—scutellum of male; *c*—*C. neaver*, Aust., scutellum of female. ($\times 250$.)

length. Oblique whorls, incomplete ventrally, of twelve to twenty hairs are present on each segment from the fourth to the twelfth; the three terminal segments each bear an incomplete basal whorl of seven or eight relatively short hairs, and short hairs scattered over the rest of the surface. Long and short spines are present on each segment from the third to the twelfth; on the thirteenth to the fifteenth segments are several small, curved spines.

Thorax strongly arched anteriorly, but not projecting over the head. Near the anterior margin, one on each side, are two small, slit-like depressions, and immediately in front of the scutellum, are two small, circular, admedian depressions. Dorsum clothed with short hairs. Scutellum (fig. 5), a transverse strip of chitin with the posterior

margin rounded centrally and somewhat concave towards the sides; bearing a few bristles and, in many species, some short hairs. Post-scutellum strongly arched, devoid of hairs, with a small, deep, central depression posteriorly.

Wings usually grey, with darker areas along the anterior border and more or less numerous pale spots on the field. Surface of the wing entirely covered with minute upright setae; longer decumbent hairs are also present, especially towards the apex and along most of the veins and folds. The wings have a beautiful iridescence when light falls on them at certain angles. The fringe is well-developed. Venation as shown in Plates VII and VIII; the first and third veins usually form two small cells, or interspaces, arranged in a figure-of-eight; the extreme base of the lower branch of the fourth vein is obsolete, but so far as can be judged, the vein bifurcates about the middle of the wing; the anterior or radio-medial cross-vein is relatively large, but is often partially obscured by a pale spot. The wing of the male is smaller and narrower than that of the female, and the decumbent hairs are less numerous.

Legs generally infuscated, often with darker knee-spots bounded on each side by a narrow whitish band; the terminal segments of the tarsus rather paler than the rest of the leg. The legs are somewhat thickly clothed with short hairs; the middle pair are longer, the hind pair stouter, than the others. Femora unarmed. Fore tibiae each armed with a short, stout, ventral spur and an oblique row of short, fine bristles at the distal extremity; middle tibiae unarmed; hind tibiae each with a short spur and two transverse rows of bristles apically—the distal row composed of four or five relatively long, stout, graded bristles, the proximal row of about twenty shorter, finer bristles. First tarsal segment twice the length of the second or longer, those of the middle legs distinctly longer and narrower, those of the hind legs stouter and more bristly, than the others; the second to the fourth tarsal segments decrease in length progressively, the fifth segment being as long or slightly longer than the fourth. Apical bristles of the first three tarsal segments of the middle legs differentiated, spine-like. Claws of all the legs equal, rather less than half the length of the fifth tarsal segment, each with a short curved hair arising from its base; claws simple in the female, divided at the tips in the male (fig. 6 e).

Empodium in the form of a minute branched hair, often difficult to detect.

Abdomen composed of nine segments clothed with short hairs. Abdomen of the male more slender than that of the female, the hypopygium conspicuous. Lamellae in the female small and rounded. Spermathecae (fig. 6) usually two in number, situated at

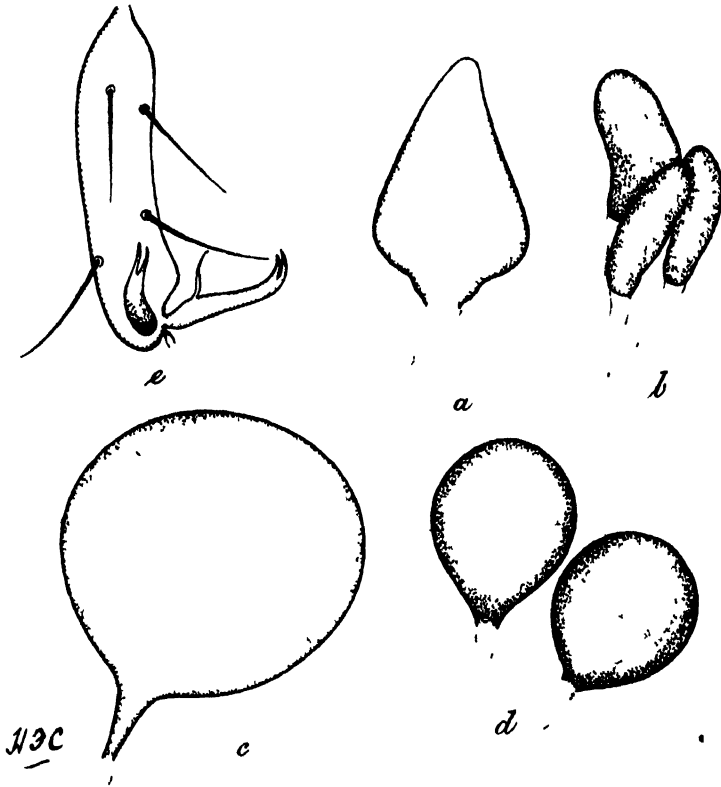


FIG 6 Spermathecae of: a—*C. distinctispennis*, Aust.; b—*C. fulvitborax*, (Aust.); c—*C. nigripennis*, sp.n.; d—*C. austeni*, sp.n. ($\times 540$); e—Claws of male *C. schultzei* ($\times 1150$)

the posterior end of the abdomen, chitinised, oval or nearly spherical in most species; the commencement of the duct is usually chitinised for a short distance.

External genitalia of the male (c.f. figs. 7 and 8). The hypopygium is relatively large and complex, easily distinguishable with the aid of a hand lens. The morphological characters present

specific differences and are of value in distinguishing closely allied species. For the structures composing the hypopygium we have adopted, in part, the nomenclature suggested by Edwards (1920). The *ninth segment* of the abdomen is shaped like an inverted coal-scuttle, the sternite being reduced to a narrow chitinous strip which is usually deeply excavated centrally, the tergite prolonged posteriorly into a broad plate which arches over the basal portions of the forceps and the intermediate appendages. The whole segment is well chitinised. The sternite is devoid of strong hairs ventrally. The tergite narrows somewhat towards its posterior end, is strongly

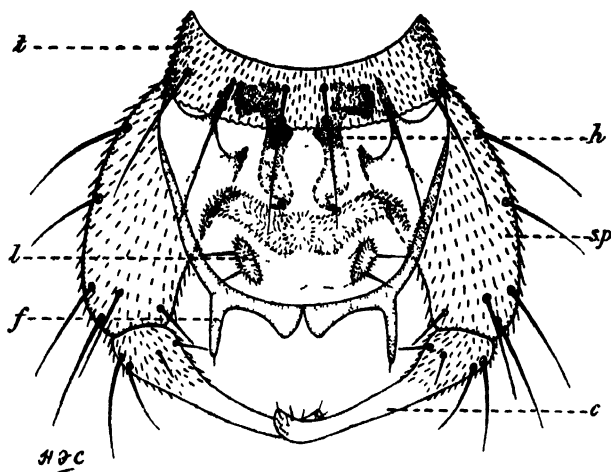


FIG. 7. *C. distinctipennis*, Aust., male hypopygium, dorsal view: *b.*, harpe; *s.p.*, side-piece; *c.*, clasper; *t.*, ninth tergite with the dorsal wall removed posteriorly; *l.*, lobe-like process of ninth tergite; *f.*, finger-like extension of posterior margin of ninth tergite. ($\times 400$.)

chitinised laterally and posteriorly, is sparsely clothed dorsally with strong hairs most numerous on the distal third, and, at its posterior margin, is frequently prolonged on each side into a finger-like structure bearing a minute hair at the apex. The lower surface of the projecting portion of the tergite is lined by membrane which bears apically two lobe-like processes studded with minute spines, intermixed with which are a few short hairs; in some positions of the hypopygium these lobes protrude prominently beyond the posterior margin of the tergite. Anterior to these processes, and situated more ventrally, is a transverse strip of the membrane

covered with spicules which appears to be intimately connected with the distal extremities of the harpes. The anus lies posterior to this strip of membrane—between the bases of the two lobe-like processes. *Forceps* well-developed, highly chitinised. Side-piece (*i.e.*, basal portion of the forceps) large, tapering slightly to the distal extremity; proximal end broad and furnished with one or two highly chitinised root-like internal projections, one of which

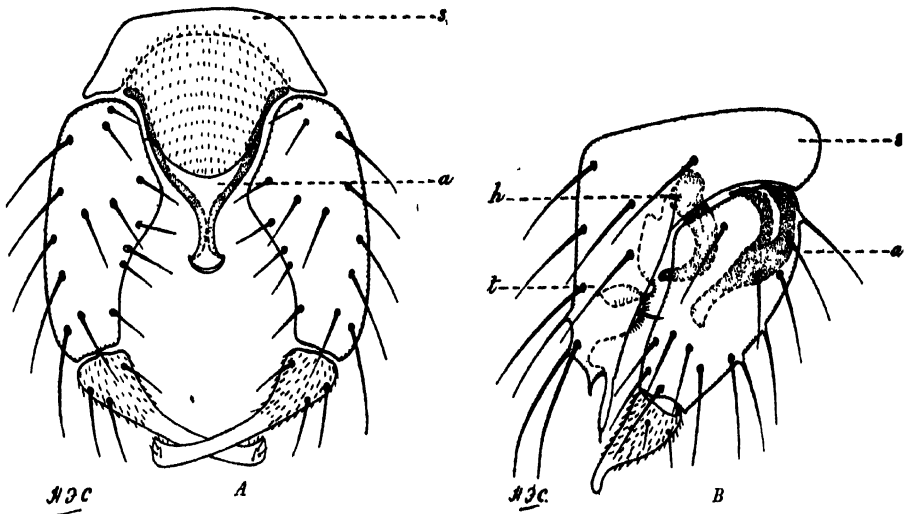


FIG. 8. *C. distinctipennis*, Aust., male hypopygium. A—ventral view. B—lateral view: *t*, ninth tergite; *s*, ninth sternite; *b*, harpe; *a*, aedocagus. ($\times 400$.)

articulates with the basal portion of the harpe of that side; clothed with strong hairs on its ventral and lateral aspects. Clasper almost as long as the side-piece with which it articulates; less highly chitinised than the side-piece, broad at the base narrowing rapidly, and ending in a slightly expanded, usually spoon-like apex; clothed at its base with minute hairs intermixed with a few longer and stouter ones, and bearing at its apex a few minute hairs. *Harpes* in the form of two chitinised admedian plates, each composed of two parts, a shorter root-like proximal portion, and a longer distal portion. The proximal portions articulate with the root-like processes at the bases of the side-pieces; they are often quite insignificant and difficult to distinguish, but sometimes appear as highly chitinised processes attached to the distal portions at right

angles. The harpes vary in shape and size in different species, and are of great value in identification. *Aedoeagus* usually somewhat Y-shaped, situated ventrally, with the stem directed backwards. The stem or distal portion is usually less highly chitinised than the limbs, and is shaped like a gutter. The limbs form a posteriorly directed arch over the ventral excavation in the ninth sternite; they are connected posteriorly and ventrally by a thin chitinous sheet—the ventral wall—which is continued anteriorly by a membrane to the ninth abdominal segment. This membrane is sometimes more or less covered with spicules.

PUPA (fig. 9). The pupa is of the usual type found in the Diptera Nematocera. It is well chitinised and is entirely free from the larval pelt. The integument is granular, and in certain areas is covered more or less densely with small squamose spines. These spines are particularly well-developed on the antero-median portion of the cephalo-thorax (operculum) and on the terminal segment of the abdomen. Tubercles bearing spines and bristles are also present, and are most numerous on the third to seventh abdominal segments. The size and structure of the tubercles are of specific value, and their general arrangement and the nomenclature adopted in this paper is shown in fig. 9 *b*.

Cephalo-thorax. The cephalo-thorax is relatively large and is sharply separated from the abdomen. It is divided by a deep dorsal depression into a cephalic and a thoracic portion; the cephalic portion is situated anteriorly and is but little depressed. The respiratory trumpets (fig. 12) are of moderate length and almost straight. They arise from tubercles situated on the antero-lateral region of the thoracic portion, each tubercle is drawn out distally into a narrow stalk-like portion or pedicle of varying length, with which the base of the trumpet articulates freely. The proximal two-thirds of the trumpets are covered with small squamose spines, and on the inner side of each are a variable number (usually two to four) of more or less conspicuous knobs. The main tracheal trunk runs through the middle of the trumpets and gives off branches to these knobs, at the end of each of which is a minute opening; it terminates in a fan-like arrangement of, usually, six similar branches. The wing- and leg-cases are attached to the sides, and extend backwards as far as the posterior border of the second abdominal segment.

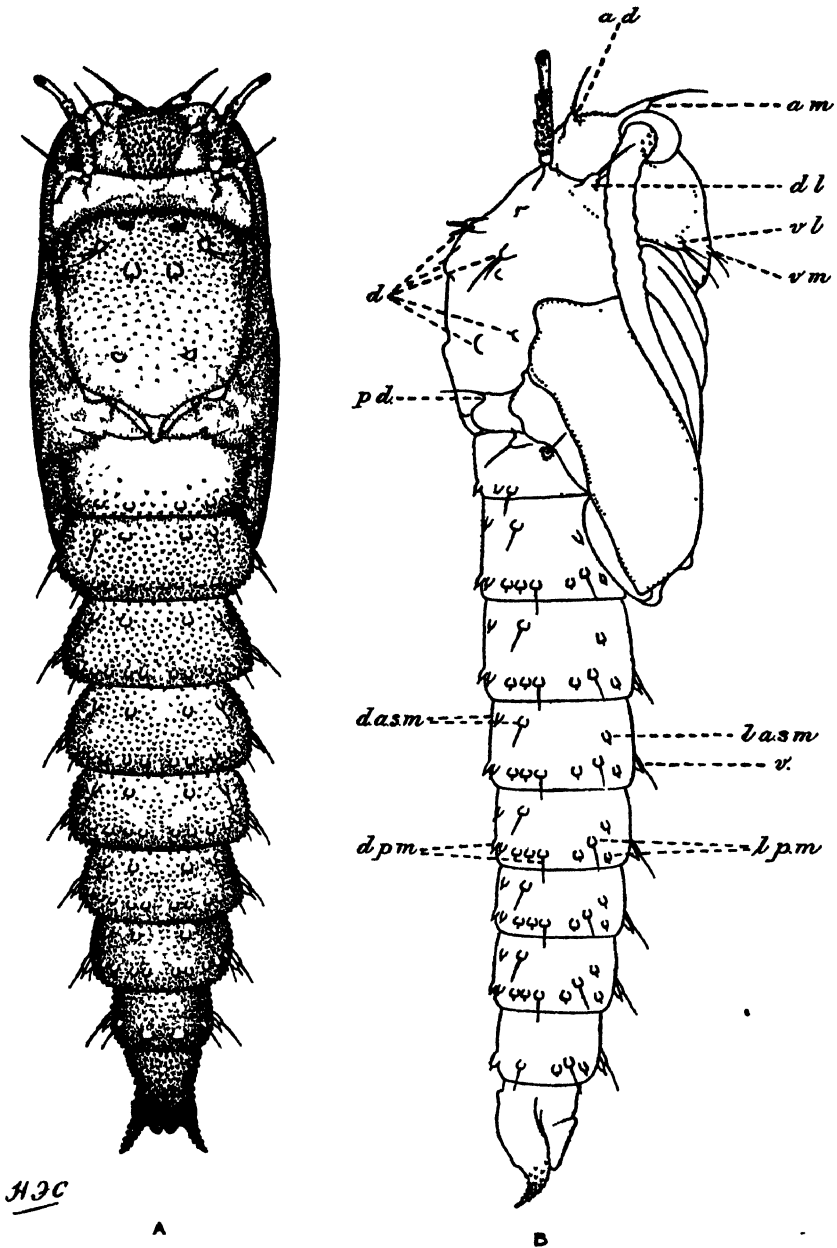


FIG. 9. Pupa of *Culicoides accraensis*, sp.n. (male). A—Dorsal view. B—Lateral view: a.m., anterior marginal tubercle; a.d., anterior dorsal tubercle; d.l., dorso-lateral tubercle; v.l., ventro-lateral tubercle; v.m., ventro-median tubercle; d., dorsal tubercles; p.d., postero-dorsal tubercle; d.a.s.m., dorsal antero-submarginal tubercles; d.p.m., dorsal postero-marginal tubercles; l.a.s.m., lateral antero-submarginal tubercle; l.p.m., lateral postero-marginal tubercles; v., ventral tubercles. ($\times 85$ circa.)

Both the cephalic and thoracic areas bear tubercles, and the following may be distinguished on each side of the body :—

Anterior-marginal. Situated anteriorly and slightly ventrally near the middle line, just internal to that part of the integument overlying the two basal segments of the antenna of the enclosed insect. It is a long conical tubercle directed laterally and bearing a moderately long stout bristle.

Anterior-dorsal. Situated slightly external and posterior to the antero-marginal tubercle and on the upper margin of the eye of the imago. It bears a short stout bristle and a minute spine.

Dorso-lateral. Situated just in front of, and external to, the base of the respiratory trumpet and dorsal to the antenna of the imago. It is a small tubercle of variable shape, and is provided with a delicate bristle and one or two minute spines.

Ventro-lateral. A somewhat inconspicuous rounded hump situated near the lower margin of the eye of the enclosed insect. Two or three delicate bristles of varying length arise from the apex.

Ventro-median. Situated near the middle ventral line of the cephalic area, immediately internal to the third segment of the palp of the adult. This tubercle is very small and inconspicuous, and may be represented only by the one or two small delicate setae which it bears.

Dorsal. A variable number of tubercles situated on the central portion of the thoracic region, above the mesothorax of the enclosed insect. Some of these tubercles are rudimentary and devoid of bristles or spines, but at least three well-developed tubercles are present on each side of the middle line. These lie above the anterior portion of the mesothorax and form a triangle with the apex pointing laterally.

Postero-dorsal. A small tubercle bearing a delicate seta, situated immediately external to the post-scutellum of the imago.

Abdomen flexible and capable of a considerable range of movement but normally extended directly backwards. It consists of nine well-marked segments, and is broad at the base, gradually narrowing towards the apex. The first is slightly shorter than the others, the last elongate and the intermediate segments sub-equal in length. The last segment terminates in two curved, divergent, lateral processes with sharply pointed tips which are directed dorsally;

these processes are merely highly developed lateral tubercles. In the male the terminal segment is furnished ventrally with cases for the forceps. The abdominal tubercles are somewhat flattened and, on segments three to seven, may be separated into dorsal, lateral (actually ventro-lateral), and ventral groups. The dorsal and lateral groups each consists of two series of tubercles, the postero-marginal and the antero-submarginal. The ventral group consists of postero-marginal tubercles only. The lateral tubercles are the most highly developed, the other tubercles become smaller the nearer they approach the middle line of the body.

Postero-marginal. The dorsal group usually consists of five tubercles, the outer three of which are sometimes slightly separated from the inner two. The lateral and ventral groups each consists of three tubercles.

Antero-submarginal. The dorsal group consists of two tubercles, the lateral group of one, which is situated almost directly over the lowest postero-marginal.

On the first, second and eighth segments the number of tubercles is reduced; the ninth segment is devoid of bristle-, or spine-bearing tubercles. The abdominal tubercles usually bear delicate setae, short stout spines, or minute spines. These setae and spines, however, are slightly variable, and some of the tubercles, especially the inner members of the dorsal postero-marginal row, occasionally appear to be unarmed.

LARVA (fig. 10). The mature larvae are long (2 to 5 mm.) and slender, and are white or creamy-white in colour with yellowish-brown heads.

Head relatively small, sub-conical, convex on the dorsal and rather flattened on the ventral aspect, bearing a few small dorsal, lateral, and ventral hairs arranged as shown in the figure.* Dorsally the head capsule is divided into three plates—a median (clypeal) and two lateral (epicranial)—by sutures, along the lines of which rupture occurs. The eyes are heavily pigmented, and each consists of a small, sometimes separate, anterior portion, and a large posterior portion; normally they are situated laterally, just behind the middle of the head, but in larvae which are approaching pupation they are retracted, and not infrequently may be observed on, or near, the

* Two pairs of minute hairs (not shown in the figure) are present near the posterior dorsal margin.

posterior margin of the head (c.f. fig. 10, 3). The antennae are represented by minute membranous structures situated near the

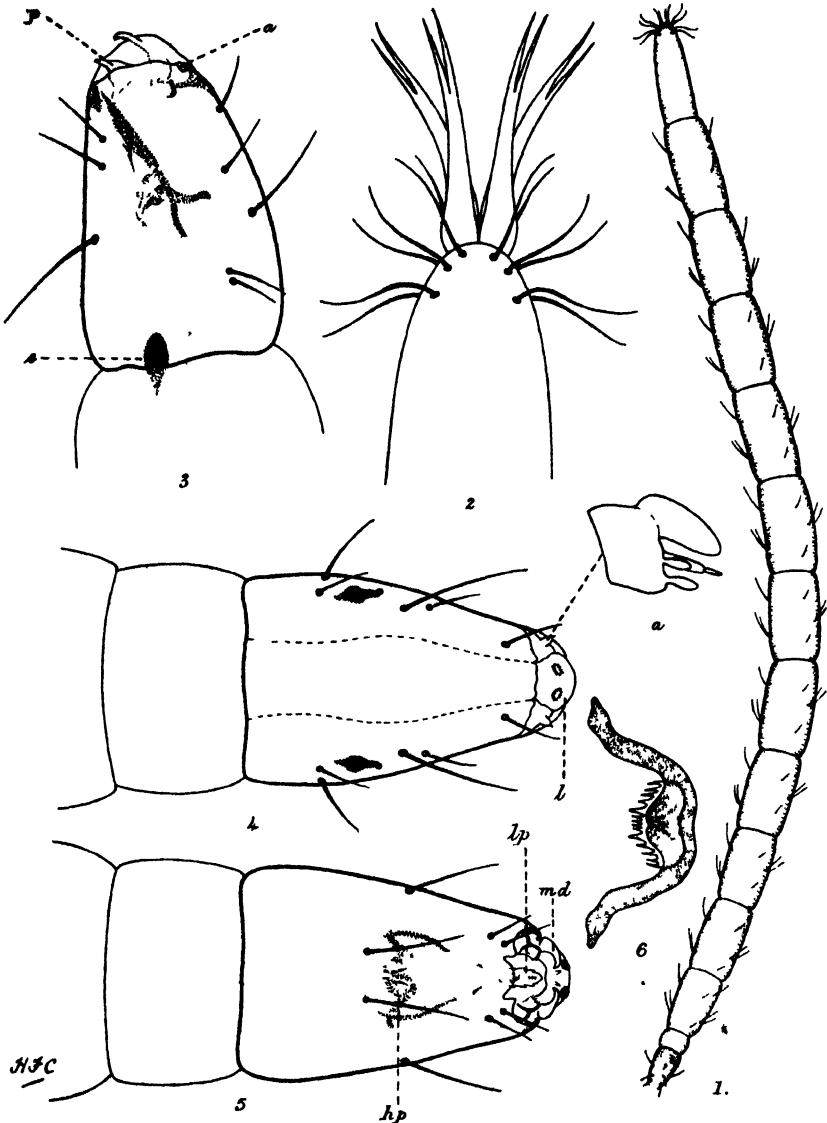


FIG. 10. *C. accraensis*, sp.n. 1—Mature larva ($\times 48$) 2—last segment with anal. gills extended ($\times 160$). 3—head, lateral view: *a.*, antenna, *p.*, palp, *e.*, eye ($\times 360$). 4—head, dorsal view: *a.*, antenna of *C. nigripennis*, sp. n. (lateral view greatly enlarged); *l.*, labrum ($\times 360$). 5—head, ventral view: *md.*, mandible, *lp*, labial plate; *bp.*, hypopharyngeal sclerite ($\times 360$). 6—hypopharyngeal sclerite (anterior teeth omitted) ($\times 930$).

anterior extremity; the apex bears a relatively large lobe and three small sub-cylindrical processes, one of which carries a delicate spine.

The lobe-like process occupies a dorsal position and, when the antenna is seen from above, almost obscures the basal portion and the remaining processes. The mouth-parts appear to be comparatively simple, but are so minute and delicate that, with the limited material available, no detailed study of them has been possible. Some idea of the relative positions and form of the more conspicuous structures, however, may be gained from fig. 10, nos. 3 to 6. Variations of specific value appear to occur in the detailed structure of the labrum, mandibles, hypopharyngeal sclerite and labial plate; but the first and last named organs are usually so difficult to observe that such differences appear to be of little practical use. The labrum is an almost semi-circular, membranous, structure projecting anteriorly, and furnished on its ventral surface (epipharynx) with a pair of small chitinous hooks, and minute papillae arranged singly and in groups; the exact arrangement and relative sizes of these papillae appear to be variable. The mandibles are slightly curved, pointed, not very strongly chitinated structures, and are occasionally provided with a tooth near the distal third. The hypopharyngeal sclerite is a complex organ, the detailed structure of which cannot be determined solely by microscopic examination of specimens mounted in carbolic acid. The number and arrangement of the teeth arising from the posterior margin, however, can be seen without great difficulty, and appear to us to afford an important means for larval differentiation; but individual variation occurs—to what extent we are at present unable to ascertain—and, therefore, too much reliance must not yet be placed upon this character. The labial plate is relatively slightly chitinated and apparently varies in shape, and in the number and size of the teeth which it bears.

Body cylindrical, composed of twelve elongate, well-differentiated, segments which bear a few delicate, inconspicuous, setae. The last segment is bluntly rounded distally, and is provided with six pairs of longer and stronger hairs confined to the posterior end. The exact arrangement of the body hairs, except those on the terminal segment, is not easily appreciable, and, in the majority of the species examined, they were not found to be of any systematic importance. Immediately behind the head is a small, but distinct, segmental-like constriction which gives the body the appearance of being composed of thirteen segments. This apparently is the neck;

it is entirely devoid of hairs, and in mature larvae the presence of imaginal buds in the three following segments, which become much swollen prior to pupation, indicates that it cannot be the first thoracic segment. Extending from the last segment are four retractile anal gills; these are elongate membranous structures each deeply cleft distally into two pointed processes.

SPECIFIC DESCRIPTIONS

Culicoides inornatipennis, sp. n.

| MEASUREMENTS. | | | | | Female. | Male. |
|--------------------------|-----|-----|-----|-----|---------|---------|
| Length of body* | ... | ... | ... | ... | 1.1 mm. | 1.0 mm. |
| Length of wing | ... | ... | ... | ... | 0.9 mm. | 0.8 mm. |
| Greatest breadth of wing | | ... | ... | ... | 0.4 mm. | 0.3 mm. |

Head dark brown. Eyes separate in both sexes, the internal chitinous thickening in the female distinct. Proboscis brown. Palpi brown, third segment moderately inflated. *Antennae* dark brown, with dark brown hairs; in the female, segments four to ten from once and a half to twice as long as broad; in the male, the thirteenth segment distinctly longer than the fifteenth. *Thorax* dark brown, with distinct greyish-brown markings as shown in fig. 22 c; sparsely clothed with brown hairs. Scutellum uniformly greyish-brown; bearing one central and two lateral bristles and a few short hairs (in the female eight or nine, in the male four). Post-scutellum dark brown. Pleurae dark brown. *Wings* (Plate VII, fig. 1) unspotted. Decumbent hairs moderately dense, most abundant on the apical anterior portion and present in more or less well-defined rows, mainly along the veins and folds, to the base of the wing; also moderately numerous in the anal angle and the fork of the fifth vein. Halteres in the female yellowish except the extreme bases of the knobs, which are brown; in the male paler, cream-coloured. *Legs* rather pale brown with indistinct light-coloured knee-spots. *Abdomen* dark brown. Spermathecae two, moderately well chitinated, oval, measuring about 40μ by 30μ ; the commencement of the duct only, chitinated.

HYPOPYGIUM (fig. 11). Relatively slightly chitinated. *Ninth segment*: posterior margin of the tergite notched in the middle,

* Unless otherwise indicated this measurement is made from specimens mounted in carboloid and is taken from the anterior margin of the thorax to the posterior end of the abdomen. *Vide* foregoing paper—Part I—p. 192.

and extended on each side into a very long and narrow (length approximately seven times the width in the middle) finger-like process. *Forceps*: side pieces and claspers normal. *Harpes* (fig. 20, *g*) simple, the distal extremities very narrow and twisted in a ventral direction; proximal portions more strongly chitinised and placed almost at right angles to the terminal portions. *Aedoeagus* Y-shaped, gradually tapering to a distal, rather broad, gutter-like process with a blunt apex; the limbs more strongly chitinised, with everted proximal extremities, forming a relatively narrow and pointed arch above the ninth sternite. The membrane connecting the ventral wall of the aedoeagus with the ninth sternite is devoid of spicules.

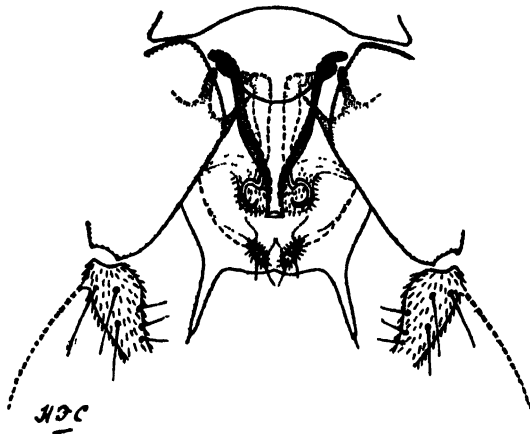


FIG. 11. *C. snornatipennis*, sp.n., male hypopygium, ventral view. ($\times 400$.)

PUPA. Length about 1.8 mm. Operculum densely covered with squamose spines. *Respiratory trumpets* (fig. 12) short and nearly straight, raised on relatively long stalks; length of the trumpet about 0.14 mm., length of the stalk about 0.05 mm. The trumpet bears three or four quite small knob-like processes, the most distal of which is situated near the centre of the anterior part. The main tracheal trunk terminates distally in a fan-like group of short blunt processes. *Cephalo-thorax*. Anterior marginal tubercle rather small, conical, bearing a relatively long, stout, spine; anterior dorsal tubercle prominent, rounded, bearing a stout spine and a minute spine; dorso-lateral tubercle small, conical, bearing a hair and a minute spine; ventro-lateral tubercle a rounded hump, bearing a

long and a moderately long hair; ventro-median tubercle very small, bearing a small hair. Dorsal tubercles: anterior double, the two parts being separate and well-developed but not large knobs, each bearing a stout spine; posterior, feebly developed, bearing a short spine; lateral bearing a hair. In front of the anterior tubercle and a little external to it is an inconspicuous unarmed tubercle. Postero-dorsal tubercle small, bearing a long hair. *Abdomen.* Anal segment with sharply pointed terminal processes. The dorsal and lateral tubercles (fig. 12) on the abdominal segments are rather

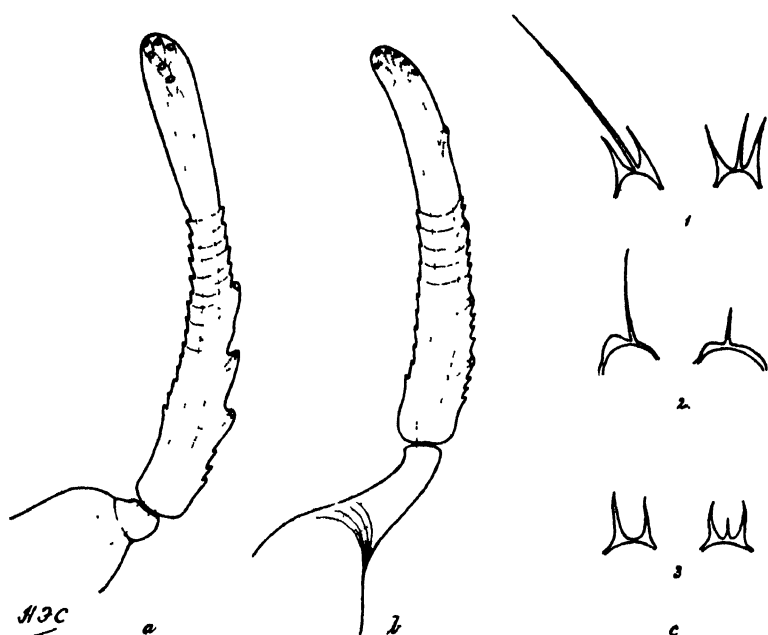


FIG. 12. *a*—Respiratory trumpet of pupa of *C. accraensis*, sp.n.; *b*—trumpet of *C. inornatipennis*, sp.n.; *c*—types of abdominal tubercles of pupa of *C. inornatipennis*, sp.n. 1—lateral; 2—ventral; 3—dorsal (postero-marginal). (*a* and *b* $\times 360$, *c* $\times 450$.)

small, each with two sharp points between which are 'the hairs or spines. Dorsal tubercles: antero-submarginal, the inner bearing a short spine and the outer a hair; postero-marginal four only in number; the inner and the outer bearing spines, the two middle tubercles apparently unarmed. Lateral tubercles larger than the others: antero-submarginal, bearing a spine; postero-marginal, the middle one bearing a hair, the other two, spines. Traces are

sometimes visible of a second, rudimentary, antero-submarginal tubercle. Ventral tubercles of normal form: the middle one bearing a hair, the other two, spines.

LARVA. The larva is of the usual *Culicoides* type, vermiform, aquatic, very active; head well chitinated, yellow; body cylindrical, almost white, very sparsely clothed with hairs, and with the usual anal appendages. Details unfortunately cannot be given, as we were not so fortunate as to find the pelt of the only larva which was isolated and successfully bred through.

HABITAT: Nsawam, Gold Coast, April, 1920. A single male reared from materials taken from a rot-hole in the stump of a silk-cotton tree (*Eriodendron anfractuosum*). From the same material were reared *C. accraensis*, sp. n., *C. punctithorax*, sp. n., *C. clarkei*, sp. n., and *C. eriodendroni*, sp. n. Two females reared from rotting materials taken from the base of a banana plant; the larvae in this case were associated with those of several species of *Dasyhelea*.

C. inornatipennis is, by reason of its unicolorous wings, one of the most easily recognised African species of *Culicoides*, being, so far as we are aware, the only member of the genus yet described from Africa in which no trace of wing markings occurs.

Culicoides fulvithorax (Aust.)

Johannseniella fulvithorax, Aust. Bull. Ent. Res., Vol. III, p. 105, 1912.

Culicoides ochrothorax, Carter. Annals Trop. Med. and Parasitol., Vol. XII, p. 298, 1919.

The above synonymy has been determined by a comparison of the types. One female only of this striking species has been obtained during this investigation and, in view of the descriptions already published, only the following characters need be noted.

MEASUREMENTS.

| | | | | | | | | |
|--------------------------|-----|-----|-----|-----|-----|-----|-----|---------|
| Length of body | ... | ... | ... | ... | ... | ... | ... | 1.0 mm. |
| Length of wing | ... | ... | ... | ... | ... | ... | ... | 1.0 mm. |
| Greatest breadth of wing | ... | ... | ... | ... | ... | ... | ... | 0.4 mm. |

The eyes are broadly contiguous dorsally. The third segment of the palp is moderately expanded and is normal, the description given by Carter apparently being made from a preparation

which had been subjected to pressure. The fourth to tenth segments of the antennae are elongate, sub-cylindrical, from two to nearly three times as long as wide. The scutellum bears three bristles, one central and two lateral, and is devoid of short hairs. The spermathecae are of unusual form and, in the single specimen examined, are three in number; they are somewhat sausage-shaped and of unequal sizes (fig. 6).

HABITAT: Accra, Gold Coast. Taken in the evening on a window of the laboratory.

Culicoides schultzei (End.)

Ceratopogon schultzei, End. Denks. Med. Ges. Jena, Vol. I, pp. 155-162, 1908.

The species which we have identified with Enderlein's *C. schultzei* from S.W. Africa agrees with the description given by that author in all essential points, notably in the complete fusion of the first and third longitudinal veins of the wing, in the spotting of the wing, and so far as can be determined from the incomplete figure given, in the structure of the male hypopygium. It apparently differs chiefly in the colouration of the thorax, which is described as deep brown with a few distinct small yellowish-grey spots; but as the colouration of the thorax varied somewhat in our specimens this difference is probably not of specific importance. The differences between *C. schultzei* and *C. kingi*, Aust., have been pointed out by Austen (1912), and need not be recapitulated here.

| MEASUREMENTS. | | | | | | Female. | Male. |
|--------------------------|-----|-----|-----|-----|-----|---------|---------|
| Length of body | ... | ... | ... | ... | ... | 1.4 mm. | 1.5 mm. |
| Length of wing | ... | ... | ... | ... | ... | 1.1 mm. | 0.9 mm. |
| Greatest breadth of wing | ... | ... | ... | ... | ... | 0.4 mm. | 0.3 mm. |

Head grey; nape and occiput dark brown, with dark hairs; frons grey; clypeus pale brown. Eyes separate, the internal⁴ chitinous thickening distinct in the female. Proboscis pale brown. Palpi brown; the third segment considerably swollen and not attenuated anteriorly; the fourth segment relatively short, distinctly shorter than the fifth. *Antennae* (fig. 4 *a* and *b*): Torus dark brown, flagellum segments brown, the basal segments (four to ten) in the female stout, ovoid, from one and one-third to one and

one-half times as long as broad; in the male the terminal segment longer than either of the two preceding segments. *Thorax* drab-grey, with many dark, sepia-coloured spots and blotches arranged as in the figure (fig. 22 *d*). The sizes of the spots and patches vary—in most specimens the thorax is predominantly grey, but in some it is predominantly dark brown; in the male the dark markings are larger and more conspicuous than in the female. Scutellum brown in the centre, grey laterally, bearing two long dark bristles in the middle and one on each side; short hairs absent. Post-scutellum dark brown. Pleurae drab-grey like the dorsum of the thorax, and similarly marbled with dark brown markings. *Wings* grey to brownish-grey, darker along the anterior border, with numerous sharply-defined pale spots as shown in the figure (Plate VII, fig. 2). The wing markings are rather variable, for example, the four spots in the apical portion of the wing are sometimes entirely separate, occasionally completely united forming a semicircular pale area, but most commonly as shown in the figure. The anterior margin bears three dark spots—the first, and darkest, a rectangular patch situated in the middle of the wing at the junction of the first and third longitudinal veins with the costa, the second, a paler and more diffuse spot, situated about midway between the first dark spot and the apex of the wing, the third, a larger but less distinct spot than either of the other two, situated towards the base of the wing. In the male (Plate VII, fig. 2 *a*) the dark spot in the middle of the anterior border is distinctly shorter and more nearly square than in the female, and the proximal pale spot longer. Venation as shown in the figure; first and third longitudinal veins *completely fused*, so that the characteristic cells usually formed by these veins are absent. Wings sparsely clothed with decumbent hairs, which are most numerous on the distal third, at the anterior margin, and along the veins. Halteres each with the apical half of the knob cream-coloured, the proximal half and stem dark. *Legs* pale brown, with dark knee-spots and pale bands on either side of these spots. Femora with dark tips, above each of which is a narrow pale band; proximal portion pale brown, becoming more dusky towards the pale band. Tibiae with bases very narrowly dark followed by a narrow whitish band, and a slightly broader dusky

band which reaches to about the middle; apical half of the tibia pale brown with the extreme apex dark brown. First tarsal segments slightly infuscated, other tarsal segments pale brown. *Abdomen* dark brown, clothed with short dark hairs. Spermathecae two, rounded or slightly longer than broad, diameter about 50μ by 45μ , dark and highly chitinised; only the extreme end, about 4μ , of the duct is chitinised.

HYPOPYGIUM (figs. 13 and 14). *Ninth segment*: sternite deeply excavated centrally; tergite relatively long and narrow posteriorly, finger-like extensions and apical lobe-like processes well-developed.

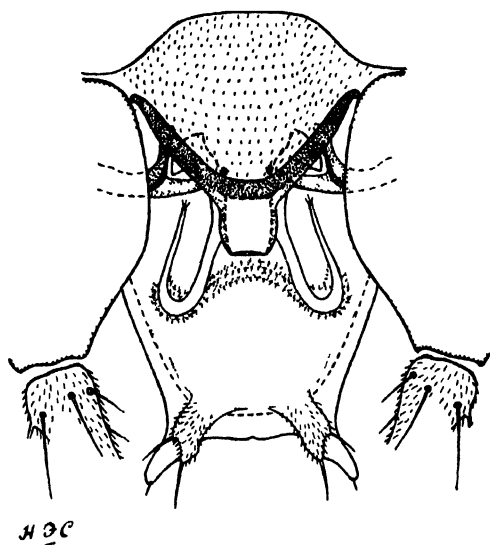


FIG. 13. *C. schultzei* (End.), male hypopygium, ventral view. ($\times 400$.)

Forceps: side-piece with two basal chitinous processes projecting inwards; clasper clothed with minute hairs for the greater portion of its length, and with some of the stronger basal hairs unusually long. *Harpes* (fig. 20h) in the form of two unbranched, long, narrow, tapering chitinous structures with sinuous outlines; they are bent ventrally, almost at a right angle, near the middle, and distally each is finely rounded and bears three or four minute hairs. *Aedoeagus* broad at the base, narrowing at its posterior third to form a straight, broad, gutter-like process directed backwards and furnished with a ventral lip of thin chitin; in ventral view this lip

is very difficult to detect, and the extremity of the aedoeagus appears to be notched.

PUPA. Length about 2 mm. *Respiratory trumpets.* Length about 0.2 mm. Distal ends darkened, middle portions closely ringed, lower portions bearing two or three knob-like processes. The main tracheal trunk terminates distally in a fan-like arrangement of about six short blunt processes. *Cephalo-thorax:* anterior

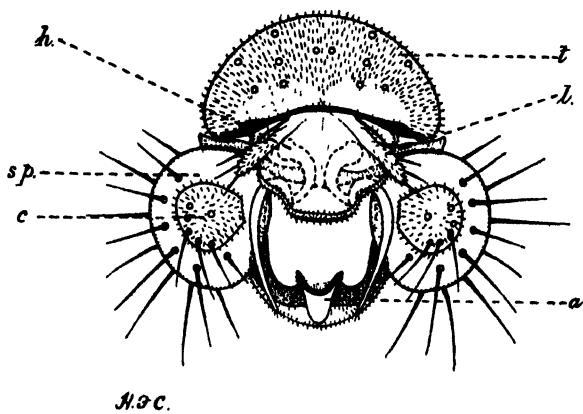


FIG. 14. *C. schultzei* (End.), male hypopygium, posterior view: *t*, ninth tergite; *l.*, lobe-like process of ninth tergite; *a.*, aedoeagus, showing ventral lip; *b.*, harpe; *s.p.*, side-piece; *c.*, base of clasper. ($\times 400$.)

marginal tubercle smaller than in *C. accraensis*, sp. n. (c.f. fig. 9), bearing a short stout spine; anterior dorsal larger, conical, bearing a short, stout, spine and a minute spine; dorsal-lateral small, bearing a short hair; ventro-lateral small, bearing a short hair; ventro-median tubercle absent, but represented by a single minute hair. Dorsal tubercles: as in *C. accraensis*. Postero-dorsal tubercle small, bearing a minute hair and, apparently, a minute spine. *Abdomen:* anal segment with squamose spines almost confined to the anterior region; tips of the terminal processes dark. Dorsal tubercles: as in *C. accraensis*, but smaller. Lateral tubercles: as in *C. accraensis*, but smaller. There is an additional tubercle, most distinct on the apical segments, situated above the central postero-marginal tubercle. Ventral tubercles: as in *C. accraensis*, but smaller.

LARVA. Length 2 mm. to 2·9 mm., average of five 2·4 mm. Greatest breadth 0·14 mm. to 0·19 mm., average 0·15 mm.

Head, length about 0·1 mm., greatest breadth about 0·09 mm. Eyes large, bilobed. Bristles extremely small, apparently arranged as in *C. accraensis*. Mental plate very slightly chitinated and difficult to see, situated rather more posteriorly than in *C. accraensis*, more or less semicircular, bearing on each side apparently one large and four small teeth. Hypopharyngeal sclerite (fig. 21 e) rather heavily chitinated, moderately large, bearing on each side three pointed processes, the middle one being large and broad. Mandibles simple, pointed. *Body* appearing almost hairless, but actually bearing a few very minute hairs; terminal hairs on the anal segment very small, but, as far as can be determined, arranged as in *C. accraensis*.

HABITAT: Accra, Odorkor and Oblogo, Gold Coast. Collected in the evening upon the windows of the laboratory from December, 1919, to April, 1920; abundant throughout this period and the species most frequently found in this situation. A few specimens taken at night in a bungalow at Accra. Larvae and pupae found at Odorkor in puddles near a stand-pipe, and at Oblogo in a back-water of the river Densu which was used as a washing-place. Also reared from rotting wood taken from canoes lying in the river Densu at Oblogo.

Culicoides punctithorax, sp. n.

| MEASUREMENTS. | | | | | | Female. | Male. |
|--------------------------|-----|-----|-----|-----|-----|---------|---------|
| Length of body | ... | ... | ... | ... | ... | 1·5 mm. | 1·5 mm. |
| Length of wing | ... | ... | ... | ... | ... | 1·1 mm. | 1·1 mm. |
| Greatest breadth of wing | ... | ... | ... | ... | ... | 0·4 mm. | 0·3 mm. |

Head dark brown, occiput brown with brown hairs. Eyes in both sexes narrowly separate, internal chitinous thickening in the female distinct. Proboscis brown. Palpi dark brown, third segment moderately swollen. *Antennae* brown; first segment in the female unusually prominent, as large as the torus, dark brown; torus dark brown; flagellum segments rather darker brown than usual. In the female, segments four to ten oval, of almost equal lengths, each about one and a half times as long as broad; segments eleven

to fifteen distinctly shorter than usual, from two to three times as long as broad; terminal segments (thirteen to fifteen) of the male antenna relatively short, particularly the last, which is distinctly shorter than the thirteenth. *Thorax* dark brown with conspicuous light grey markings on which are numerous small dark spots, as shown in fig. 22 *b*. Scutellum dark brown with greyish lateral areas, bearing two central and two lateral bristles and a number (twelve or thirteen) of short hairs. Post-scutellum dark brown. Pleurae dark brown. *Wings* grey, rather darker along the anterior border, the middle part of which is the darkest portion of the wing. Venation and pale markings arranged as shown in the figure (Plate VII, fig. 4). Wings thickly clothed with decumbent hairs. Halteres with cream-coloured knobs, the stems pale brown. *Legs* brown, tarsal segments rather paler; there are pale bands, less distinct in the male, on each side of the dark knee joints of all the legs, those on the bases of the tibiae being the more distinct. *Abdomen* dark brown, with brown hairs. Spermathecae two, rather highly chitinised, oval, measuring about 48μ by 38μ ; the chitinised commencement of the duct short and conical.

HYPOPYGIUM (fig. 15). *Ninth segment*: posterior margin of the tergite notched centrally and bearing on each side a finger-like extension of great length—from eight to nine times as long as the width in the middle. *Forceps*: side-piece stout, clothed laterally with rather longer and stronger hairs than usual; distal extremity of the clasper rounded, with a somewhat irregular margin. *Harpes* (fig. 20 *f*) simple, rather strongly chitinised; proximal portion more strongly chitinised and directed laterally at a right angle to the distal portion, the latter broad basally, tapering posteriorly, with the apical fourth bent ventrally. *Aedoeagus* forming a rather narrow arch over the depression in the ninth sternite, tapering gradually to a relatively broad, straight, distal portion; the extremity consists of a larger central lobe and two small lateral lobes, the central lobe is an extension of the floor of the gutter and is apparently analogous to the ventral lip seen in *C. schultzei* (fig. 14), although less well-developed. The membrane connecting the chitinised ventral wall of the aedoeagus with the ninth sternite is devoid of spicules.

PUPA. Length about 2.4 mm.; operculum covered with dense

sharp-pointed squamose spines. *Respiratory trumpets* arise from relatively long stalks; length about 0.2 mm. They are of nearly the same diameter throughout, and bear two or three very small blunt knobs. The main tracheal trunk terminates distally in a fan-like arrangement of about six short blunt processes. *Cephalo-thorax*: Anterior marginal tubercle large, conical, bearing a very strong, stout, spine which is short or of moderate length; anterior dorsal large, conical, bearing a stout hair and a short spine; dorso-lateral bearing a long hair and a short spine; ventro-lateral large, rounded, bearing a long and a short hair;

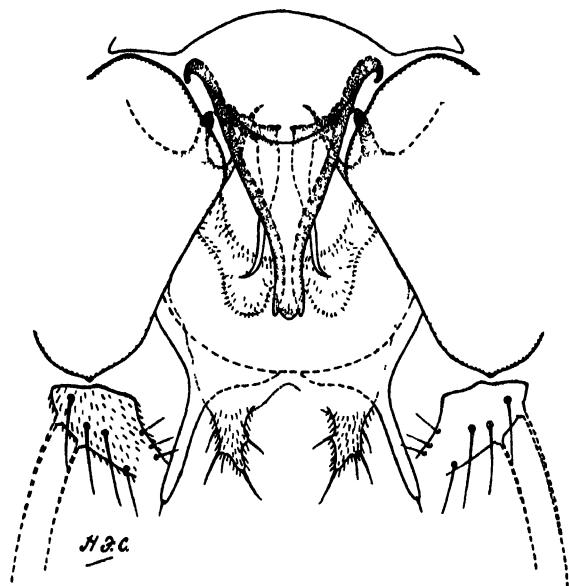


FIG. 15. *C. punctithorax*, sp.n., male hypopygium, ventral view. ($\times 400$.)

ventro-median very small but quite definite, bearing a long and a very short hair. Dorsal tubercles: anterior, the largest, bearing a short stout spine, posterior bearing a long hair, lateral bearing a long hair; behind the posterior is a small tubercle bearing a short spine; in front of the anterior, and rather lateral to it, is a rounded unarmed hump. Postero-dorsal tubercle bearing a long hair; behind it and nearer to the middle line is a small unarmed tubercle. *Abdomen*: Anal segment normal; the other segments furnished with moderately well-developed tubercles. Dorsal

tubercles: antero-submarginal, the inner bearing a relatively long spine, and the outer a long hair; postero-marginal, the outermost bearing a long hair, the second a stout spine, and the inner three minute spines. Lateral tubercles: antero-submarginal, bearing a stout spine; postero-marginal, the middle one bearing a long hair, the other two stout spines. Ventral tubercles: the middle one bearing a long hair, the other two short spines.

LARVA. The larva of this midge has not yet been identified.

HABITAT: Nsawam, Gold Coast, a station about twenty-five miles north of Accra, at the edge of the thick forest region. Reared from materials collected in a rot-hole in the stump of a silk-cotton tree (*Eriodendron anfractuosum*); from the same material *C. accraensis*, *C. clarkei*, and *C. eriodendroni* were also reared. March and April, 1920.

C. punctithorax somewhat resembles *C. schultzei* in its thoracic adornment, and is the only other *Culicoides* we have encountered in which the thorax is conspicuously spotted; it is, of course, easily separable from *C. schultzei* by its venation and wing markings.

Culicoides distinctipennis, Aust.

Culicoides distinctipennis, Aust. Bull. Ent. Res., Vol. III, p. 101, 1912.

The colour markings of the female of this species have been given in detail by Austen. With this author's description of them our specimens, both males and females, agree, and therefore no further references need be made.

| MEASUREMENTS. | | | | | | Female. | Male. |
|--------------------------|-----|-----|-----|-----|-----|---------|---------|
| Length of body | ... | ... | ... | ... | ... | 1.1 mm. | 1.1 mm. |
| Length of wing | ... | ... | ... | ... | ... | 1.0 mm. | 0.9 mm. |
| Greatest breadth of wing | ... | ... | ... | ... | ... | 0.4 mm. | 0.3 mm. |

The eyes of the female are narrowly separate above, the internal transverse chitinous thickening being distinct (fig. 1 *a*); in the male the eyes are more widely separate dorsally (fig. 1 *b*), their opposing edges not parallel, and the distance between them at the vertex greater. Second and third palpal segments (fig. 3 *b*) sub-equal, each about twice the length of any of the other three; the third segment

moderately inflated. Antennal segments four to ten in the female sub-spherical to oval ($1-1\frac{1}{2} \times 1$); the thirteenth antennal segment of the male (fig. 4 *c*) slightly longer than either of the two succeeding segments. Scutellum in the female bearing two central and two lateral bristles and six or seven short hairs; in the male with bristles as in the female but only two short hairs. Wings with pale spots as shown by Austen; venation normal. The wing of the male is smaller and narrower than that of the female; the decumbent hairs less numerous but extending as far towards the base of the wing. Spermatheca single (fig. 6 *a*), not very highly chitinised in any part and quite delicate at its distal end, shaped like a peg-top, the duct wide and chitinised for only a very short distance at its commencement.

HYPOPYGIUM (figs. 7 and 8). *Ninth segment*: sternite with the excavation moderately deep; tergite well-developed, sparsely clothed dorsally with strong hairs, most numerous on the distal third, posterior margin notched in the middle and produced on each side into a finger-like structure bearing a minute hair at its apex. Apical lobe-like processes well-developed, the transverse strip of spiculated membrane conspicuous, folded laterally, the folds often appearing at first sight as long, narrow, pointed spiculated lobes. *Forceps*: side-pieces of the usual form; claspers rather more highly chitinised than usual, clothed with minute hairs intermixed with a few longer, stouter, ones on the proximal third, and terminating in a depressed and shallow spoon-like apex bearing a few minute hairs. *Harpes* (fig. 20 *d*): in the form of two unbranched, short, stout, highly chitinised, admedian plates each with the proximal or basal portion expanded, and the distal portion short, terminating in a sharply pointed hook with the point directed laterally. *Aedoeagus* Y-shaped; the stem or distal portion short and broad, less highly chitinised than the limbs and ending in a broad mushroom-shaped structure; the limbs slightly everted basally, forming an arch over the depression in the ninth sternite which is low and wide with the apical angle rounded. The membrane connecting the arch of the aedoeagus with the ninth abdominal segment is covered with spicules.

HABITAT: Accra, Gold Coast. Collected in the evening upon the windows of the laboratory, December, 1919, to April, 1920.

Culicoides praetermissus, sp. n.

This species is very closely allied to *C. distinctipennis*, Aust., and is described from a single male which had been preserved in alcohol together with several examples of *C. distinctipennis*. In due course an examination of the hypopygium of this specimen revealed its distinctiveness, and although we are unable to give any details of the ornamentation of the thorax, the wing markings remained visible and showed slight differences which will probably enable both sexes of the species to be identified without great difficulty.

MEASUREMENTS.

| | | | | | | | | |
|--------------------------|-----|-----|-----|-----|-----|-----|-----|----------|
| Length of body | ... | ... | ... | ... | ... | ... | ... | 1.0 mm. |
| Length of wing | ... | ... | ... | ... | ... | ... | ... | 0.75 mm. |
| Greatest breadth of wing | ... | ... | ... | ... | ... | ... | ... | 0.3 mm. |

Eyes separate, much as in *C. distinctipennis*. Thirteenth and fifteenth antennal segments sub-equal. Scutellum bearing two central and two lateral bristles, and two short hairs immediately above the central pair of bristles. Wing venation and markings as shown in Plate VII, fig. 3. The markings are very similar to those of *C. distinctipennis* but differ chiefly in the presence of an additional small, pale, spot situated just beyond and below the conspicuous spot at the end of the costa, and apparently in the smaller size of the pale spot situated immediately below the junction of the interspaces formed by the first and third veins. Decumbent hairs moderately numerous and extending almost to the base of the wing.

HYPOPYGIUM (fig. 16). The structure of the hypopygium differs from that of *C. distinctipennis* as follows:—*Ninth segment*: posterior margin of the tergite less deeply notched in the middle line, and the lateral finger-like extensions relatively shorter. *Harpes* (fig. 20 k) with the distal portion longer and narrower, and the apical third bent sharply in a ventral direction. *Aedoeagus* tapering more gradually, the arch formed by the limbs being distinctly more pointed, and the ventral wall prolonged anteriorly for a greater distance. The membrane connecting the aedoeagus

with the ninth sternite clothed with spicules on its anterior two-thirds.

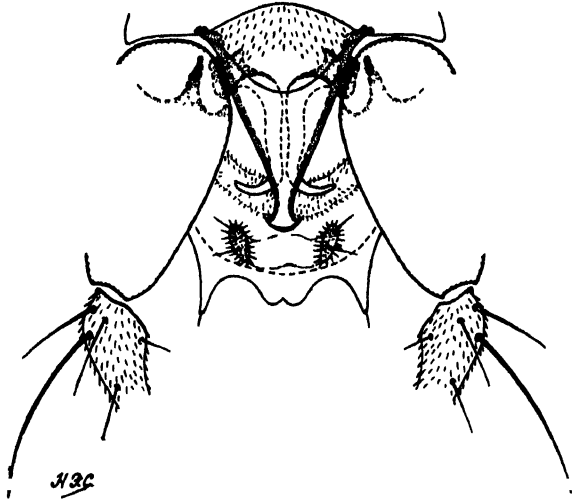


FIG. 16 *C. praetermissus*, sp.n., male hypopygium, ventral view. ($\times 400$.)

HABITAT: Accra, Gold Coast. Collected in the evening upon a window in the laboratory.

Culicoides accraensis, sp. n.

| MEASUREMENTS. | | Female. | Male. |
|--------------------------|--------|---------|---------|
| Length of body | | 1.2 mm. | 1.1 mm. |
| Length of wing | | 1.1 mm. | 1.0 mm. |
| Greatest breadth of wing | | 0.4 mm. | 0.3 mm. |

Head, occiput dark brown, clothed with short, dark, hairs; clypeus and proboscis dark brown, hairy. Eyes separate in both sexes; internal chitinous thickening in female well-developed. Palpi (fig. 3 c) dark brown; third segment very strongly inflated, fourth segment small, shorter than the fifth. *Antennae*: torus brown, flagellum segments paler; antennal segments four to ten in the female from about one and one-third to twice as long as broad; plumes of the male greyish, the last three antennal segments sub-equal, the thirteenth segment slightly the longest. *Thorax* dark brown, with two broad, admedian, dusky-white stripes which

expand posteriorly to cover the depressed area in front of the scutellum; near the centre of this area are two relatively large rounded black spots and laterally, above the wing roots, are two curved whitish patches (fig. 22 *e*). In the male the thoracic ornamentation is very indistinct, and in some specimens little trace of it can be seen. Thorax sparsely clothed with dark brown hairs. Scutellum in both sexes dark, sepia-coloured, bearing two central and two lateral bristles and several short hairs. Post-scutellum dark, sepia-coloured. Pleurae uniformly dark brown. *Wings* grey or brownish-grey, slightly darker along the anterior border, with a number of pale spots arranged as shown in the figure (Plate VII, fig. 5). The anterior margin bears one decidedly dark spot covering the terminations of the first and third veins, and a more diffuse dark spot on the distal side of the single pale spot which reaches the costal border. Venation as shown in the figure; the two small cells formed by the first and third veins distinct. Wings densely clothed with decumbent hairs which are most numerous on the distal and anterior parts; in the male these hairs are more scanty. *Halteres* brownish-yellow, the distal portion of the stems darker brown. *Legs* brownish, femora and tibiae slightly infuscated, with darker knee-spots which have on the distal (tibial) side on all the legs, and on the proximal (femoral) side on the fore legs, a narrow whitish band. *Abdomen* dark brown, clothed with dark hairs. In freshly emerged specimens there are, on the ventral aspect, three lighter coloured longitudinal stripes, a median and two lateral. Spermathecae usually two in number, but occasionally a very small third one is present; not very highly chitinised, oval, in one instance measuring 42μ by 38μ ; a small part of the commencement of the duct is chitinised.

HYPOPYGIUM (fig. 17). *Ninth segment*: sternite with a rather shallow ventral excavation; tergite relatively narrow posteriorly, with well-developed finger-like extensions and apical lobe-like processes, the posterior margin not notched. *Forceps*: side-pieces and claspers normal, similar to those of *C. distinctipennis* (fig. 7). *Harpes* (fig. 20 *a*) large broad structures, branched; the ventral branch in the form of a short rounded knob, the dorsal branch a longer structure, at first directed dorsally, then arching over the ventral branch and ending in a broad, ventrally directed, blade with

five to seven filiform processes on its posterior edge. *Aedoeagus* large, Y-shaped, very highly chitinised; the narrow, distal portion extends backwards as a gutter-like process with the apex bent slightly in a ventral direction; the limbs of the arch, as seen in a ventral view, show incurved proximal extremities and wide lateral thickenings with highly chitinised edges, the inner of which are looped near the apex of the arch and project beyond the outer edges as pointed processes. The membrane connecting the aedoeagus with the ninth sternite is without spicules.

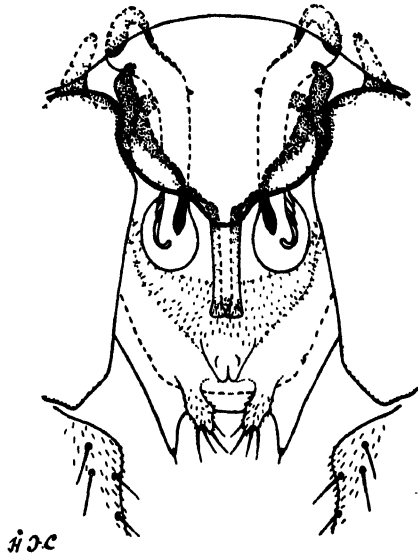


FIG. 17. *C. accraensis*, sp.n., male hypopygium, ventral view. ($\times 400$.)

PUPA. Length 1.6 mm. to 2.4 mm., average of five 2.1 mm. *Respiratory trumpets* as shown in fig. 12. Length about 0.2 mm. The trumpets are narrow in the middle thirds, and slightly dilated at the free ends; they bear two to four blunt knobs. The main tracheal trunk terminates distally in a fan-like arrangement of about six short, blunt, processes. *Cephalo-thorax*. Anterior marginal tubercle, large, conical, bearing a long stout bristle or spine; anterior dorsal, large, conical, bearing a long bristle and a short spine; dorso-lateral, bearing one long and one or two short bristles; ventro-lateral, irregularly shaped, bearing one long and one or two short bristles; ventro-median, very small, bearing a

long and a short hair. Dorsal tubercles: anterior, double, each half bearing a short stout spine; posterior, large and flat, bearing a very short, stout, spine; lateral, bearing a long and a short hair. Other dorsal tubercles, apparently variable, may also be present and are sometimes armed with very minute spines. Postero-dorsal tubercle, small, bearing a single long hair. *Abdomen*: anal segment with pointed processes nearly half as long as the segment. Dorsal tubercles: antero-submarginal, the inner bearing a short spine and the outer a long hair; postero-marginal, the outermost bearing a long hair, the second a small spine, and the inner three minute spines. Lateral tubercles: antero-submarginal, bearing a short spine; postero-marginal, the middle one bearing a long hair, the other two short spines. Ventral tubercles: the middle one bearing a long hair, the other two short spines.

LARVA. Length 2·4 mm. to 3·2 mm., average of seven 2·8 mm. Greatest breadth 0·12 mm. to 0·18 mm., average 0·14 mm.

Head, length about 0·1 mm., greatest breadth 0·08 mm. Eyes small, bilobed or kidney-shaped. Bristles as shown in fig. 10; on the ventral surface one pair, large, admedian, central, two pairs, small, admedian, anterior, and one pair, large, ventro-lateral; on the dorsal surface one pair, small, admedian, anterior, two pairs (one large and one small) central, dorso-lateral, two pairs, small, posterior, dorso-lateral, and two minute pairs admedian, posterior (see footnote, p. 224). Mental plate delicate, with a large terminal tooth and three small teeth on each side. Posterior margin of hypopharyngeal sclerite bearing, on each side, two or three small, one large, and four to six small teeth or processes (fig. 21 *a*). Mandibles simple, pointed, without teeth. *Body*: some of the body bristles are relatively large. Anal segment bearing at its posterior end six pairs of rather long and strong bristles, arranged in two groups of three bristles on each side.

HABITAT: Accra, Nsawam, Odorkor, and Oblogo, Gold Coast. Reared from larvae found in rot-holes in flamboyant trees (*Poinciana regia*), cotton trees (*Eriodendron anfractuosum*), cashew trees (*Anacardium occidentale*), *Cynometra* sp. (probably *C. megalophylla*) and other trees; a few also collected in the evening on the windows of the laboratory at Accra. December, 1919, to April, 1920.

Culicoides neavei, Aust.

Culicoides neavei, Aust. Bull. Ent. Res., Vol. III, p. 102, 1912.

A considerable number of specimens of this species, females of which have previously been recorded from Uganda and the Anglo-Egyptian Sudan, were collected. These specimens have been compared with, and found to be similar to, examples of *C. neavei* kindly sent to one of us by Mr. H. H. King from M'Volo, Anglo-Egyptian Sudan, from which locality examples are referred to by Austen in his original description. Additional characters, including the more important differential points of the male, are given.

| MEASUREMENTS. | | | | | | Female. | Male. |
|--------------------------|-----|-----|-----|-----|-----|---------|----------|
| Length of body | ... | ... | ... | ... | ... | 1.3 mm. | 1.3 mm. |
| Length of wing | ... | ... | ... | ... | ... | 1.1 mm. | 1.0 mm. |
| Greatest breadth of wing | ... | ... | ... | ... | ... | 0.4 mm. | 0.35 mm. |

The eyes are separate as in *C. distinctipennis*, Aust.; in the female with little indication of the internal transverse thickening. The third segment of the palp is moderately inflated. In the female the fourth to the tenth antennal segments are sub-spherical to oval, the length varying from nearly one and a half to almost twice the width; in the male the thirteen and fifteenth segments are of almost equal length. Scutellum (fig. 5 c) with two central and two lateral bristles and a few short hairs. We are unable to agree with Austen regarding the differences in venation between *C. neavei* and *C. distinctipennis*, when he states that in the former species the anterior cross-vein is more oblique, the bifurcation of the fourth vein nearer the base of the wing, and the shape of the interspace between the distal portions of the first and third veins different. Spermathecae two, dark coloured, moderately highly chitinised; nearly spherical, the diameter about 45μ ; duct narrow, chitinised for only a short distance, about 4μ , from its commencement.

HYPOPYGIUM (fig. 18). *Ninth segment*: the ventral depression in the sternite is deep, semicircular; the tergite of about the usual size, the apical lobe-like processes prominent, the posterior margin with well-developed finger-like processes and very slightly notched centrally with a line running from the notch in an anterior direction to about the middle. *Forceps*: side-pieces normal, claspers bluntly rounded distally, the hairs at the tip being rather

longer than usual. *Harpes* (fig. 20i) in the form of two long, narrow, unbranched chitinous processes; the distal portions each with an almost straight, posteriorly directed basal part, and a slightly spiral apical part which tapers to a filiform end and bears a few minute hairs; the proximal portions highly chitinised, laterally directed, structures articulating with the distal portions of the harpes and the roots of the side-pieces. *Aedoeagus* with the distal portion short and stout, gutter-like, not very highly chitinised, ending abruptly and with a small ventral lip; the

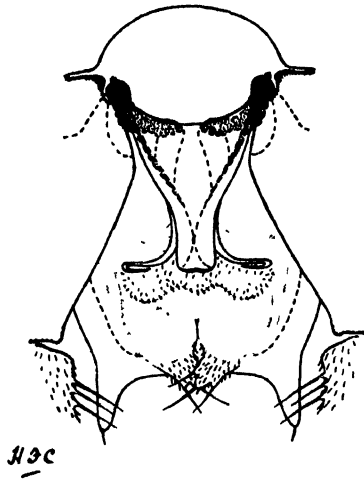


FIG. 18. *C. neavei*, Aust., male hypopygium, ventral view. ($\times 400$.)

proximal portion more highly chitinised, with the basal extremities everted; the arch formed by the limbs is broad and pointed. The ventral wall is chitinised and the membrane connecting it with the ninth abdominal segment is devoid of spicules.

HABITAT: Accra, Gold Coast. Collected in the evening upon the windows of the laboratory, January to April, 1920.

Culicoides clarkei, sp. n.

| MEASUREMENTS. | | | | | | Female. | Male. |
|--------------------------|-----|-----|-----|-----|-----|---------|---------|
| Length of body | ... | ... | ... | ... | ... | 1.1 mm. | 1.2 mm. |
| Length of wing | ... | ... | ... | ... | ... | 1.0 mm. | 1.0 mm. |
| Greatest breadth of wing | ... | ... | ... | ... | ... | 0.4 mm. | 0.3 mm. |

Head dark brown, occiput dark brown with dark hairs. Eyes narrowly separate, internal chitinous thickening distinct.

Proboscis brown. Palpi brown, the third segment moderately swollen. *Antennae* brown, torus dark brown, flagellum segments paler brown. In the female, the basal segments (four to ten) of the flagellum vary from a little over once to twice as long as broad, the first three or four being broadly oval or sub-spherical; in the male, the thirteenth segment is distinctly longer than the fifteenth. *Thorax* brown, with somewhat indistinct paler brown markings as shown in fig. 22 *a*. Dorsum sparsely clothed with dark brown hairs, which are longest posteriorly. Scutellum dark brown centrally, paler brown laterally; bearing two admedian and two lateral bristles and a few short hairs. Post-scutellum dark brown. Pleurae dark brown. *Wings* grey, with pale coloured spots as shown in the figure (Plate VII, fig. 6). The first pale spot near the middle of the anterior border of the wing envelopes the cross-vein and the proximal portion of the first interspace; its continuation from the first vein to the costa is not clearly defined in some specimens. The wings are relatively densely clothed with decumbent hairs. Halteres with cream coloured or yellowish knobs. *Legs* brown, almost uniformly coloured; fore femora with apical pale bands, middle and hind femora without distinct pale bands; tibiae of all the legs with a rather lighter coloured band upon the bases. *Abdomen* dark brown in dried specimens; scantily clothed with dark hairs which are longest and most numerous on the distal segments. Spermathecae two, dark and highly chitinised; oval or sub-spherical; the duct chitinised for only a very short distance at its commencement.

HYPOPYGIUM (fig. 19). *Ninth segment*: tergite rather broad posteriorly, posterior margin notched in the middle and ending on each side in a well-developed finger-like process. *Forceps* of the usual form. *Harpes* (fig. 20 *e*) simple, long structures, with a sinuous outline, tapering gradually to a thread-like termination, the basal portion with a thickened knob at its proximal end, and forming an obtuse angle at its junction with the terminal portion; the latter is bent ventrally. *Aedoeagus* Y-shaped; stem rather short and slender, not highly chitinised, with a blunt, rounded end; limbs narrow and highly chitinised, in ventral view slightly everted at their bases, forming a rather pointed arch. Ventral wall not chitinised, not differentiated from the membrane joining it to the ninth sternite which is not studded with spines.

PUPA. Length 1·5 mm. to 1·7 mm., average of three 1·6 mm. *Respiratory trumpets* similar to those of *C. accraensis*. Length about 0·17 mm. The trumpets bear two or three blunt knobs. *Cephalo-thorax*: spines on operculum less numerous posteriorly. Tubercles similar to those of *C. accraensis*. Anterior marginal tubercle bearing a long bristle; anterior dorsal, bearing a long hair and minute spine; dorso-lateral, bearing a long and a short hair; ventro-lateral, bearing a long and a rather shorter hair; ventro-median, very small, bearing a minute hair. Dorsal tubercles: anterior single, bearing a short, stout, hair or a long, pointed spine; posterior, bearing a long hair; lateral, bearing a long hair

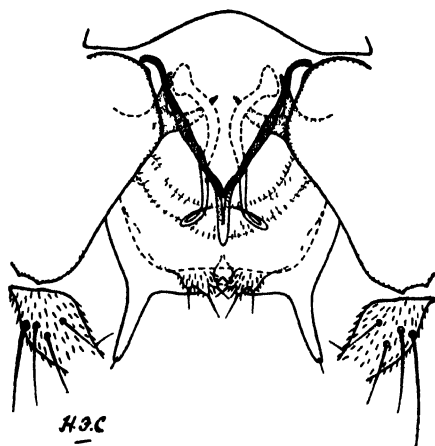


FIG. 19. *C. clarkei*, sp.n., male hypopygium, ventral view. ($\times 400$.)

Of subsidiary dorsal tubercles, mention need be made of one only—this is situated just below the posterior tubercle and slightly nearer the middle line, it is inconspicuous and bears a minute spine. Postero-dorsal tubercle small, bearing a long hair. *Abdomen*: anal segment as in *C. accraensis*; squamose spines on the dorsal surface not very dense, especially posteriorly. Dorsal tubercles: antero-submarginal, each bearing a longish hair; postero-marginal, the outermost bearing a hair, the second a long stout spine, and the inner three minute spines. Lateral tubercles very large: antero-submarginal bearing a long, strong, spine; postero-marginal, the middle one bearing a delicate seta, the other two long, strong, spines. Ventral tubercles: the middle one bears a hair, the other two rather long spines.

LARVA. Length about 3·4 mm., greatest breadth about 0·15 mm.

Head, length about 0·1 mm, greatest breadth about 0·08 mm., anterior end rather blunt. Eyes rather large, bilobed or reniform. Bristles as in *C. accraensis* but rather smaller. Mental plate somewhat similar to that of *C. accraensis*, with a terminal tooth, and four teeth on each side. Hypopharyngeal sclerite (fig. 21 d) large, moderately strongly chitinised, with four teeth on each side posteriorly. Labrum with two of the papillae very long. Mandibles with a small sub-central tooth. **Body**. bristles conspicuous, as in *C. accraensis*.

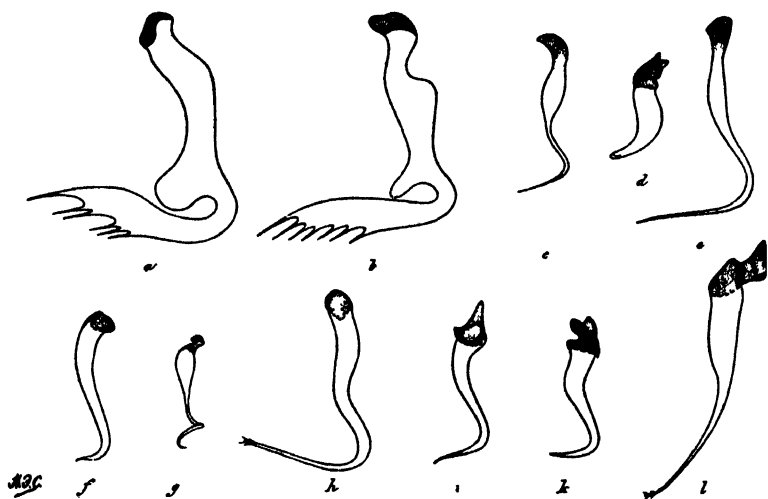


FIG 20 Lateral views (the distal extremities directed ventrally) of harpes of —
a—*C. accraensis*, sp n, *b*—*C. similis*, sp n, *c*—*C. citreus*, sp n, *d*—*C. distinctipennis*,
 Aust, *e*—*C. clarkii*, sp n, *f*—*C. punctisborax*, sp n, *g*—*C. inornatipennis*, sp n,
h—*C. schultzei* (End), *i*—*C. neavei*, Aust, *k*—*C. praetermissus*, sp n, *l*—*C. austeni*,
 sp n (× 365).

HABITAT Accra, Gold Coast, collected in the evening upon the windows of the laboratory. Nsawam and Oblogo, Gold Coast, reared from materials obtained from rot-holes in the stump of a silk-cotton tree (*Eriodendron anfractuosum*), *Cynometra* sp (probably *C. megalophylla*), and in another tree. December, 1919, to April, 1920.

We have pleasure in dedicating this species to Dr. P. S. Selwyn-Clarke, to whom we are indebted for much assistance in procuring material for this investigation.

Culicoides confusus, sp. n.

This insect, of which we possess a single female, very greatly resembles *C. clarkei*, and, indeed, was originally included among some examples of that species in our collections on account of its almost identical colour markings. Subsequently certain morphological differences were observed which, we think, warrant its separation as a distinct species. Only the chief differences between this species and *C. clarkei* are here given.

MEASUREMENTS.

| | | | | | | | |
|--------------------------|-----|-----|-----|-----|-----|-----|---------|
| Length of body | ... | ... | ... | ... | ... | ... | 1.1 mm. |
| Length of wing | ... | ... | ... | ... | ... | ... | 0.9 mm. |
| Greatest breadth of wing | ... | ... | ... | ... | ... | ... | 0.4 mm. |

Head, eyes narrowly separate, but internal chitinous thickening very indistinct. Palpi shorter than in *C. clarkei*, the third segment relatively broader. *Antennae* shorter; basal joints (four to ten) less elongate, about one and a half times as long as broad. *Thorax* with markings forming a pattern and with colouration very similar to that of *C. clarkei*, but the pale admedian stripes appear to expand anteriorly forming a narrow band round the margin, and the pale lateral stripes are broader. *Wings* (Plate VIII, fig. 4) with the pale areas in the central region, *i.e.*, between the fourth and fifth veins, more extensive; the pale spot covering the anterior cross-vein extending over the greater part of the first interspace; the median dark spot at the end of the first and third veins smaller. The wings are much less densely clothed with hairs, especially at the base between the fourth and fifth veins and posteriorly.

HABITAT: Nsawam, Gold Coast; reared from material obtained from a rot-hole in a silk-cotton tree (*Eriodendron anfractuosum*).

Culicoides eriodendroni, sp. n.

MEASUREMENTS.

Female.

| | | | | | | | |
|--------------------------|-----|-----|-----|-----|-----|-----|---------|
| Length of body | ... | ... | ... | ... | ... | ... | 1.2 mm. |
| Length of wing | ... | ... | ... | ... | ... | ... | 1.0 mm. |
| Greatest breadth of wing | ... | ... | ... | ... | ... | ... | 0.4 mm. |

Head brown, occiput brown with dark hairs. Eyes very narrowly separate, in some specimens almost meeting in the middle

line; the internal chitinous thickening present. Proboscis brown. Palpi brown; third segment much swollen. *Antennae*: torus dark brown, flagellum segments brown with brown hairs. Seven basal segments (four to ten) of the flagellum elongate, the breadth from one and a half to nearly two and a half times the length. *Thorax* uniformly brown in dried specimens, though traces may be seen of two paler, admedian stripes and lateral markings, which are quite distinct in fresh specimens; clothed with rather long and short brown hairs. Scutellum brown; bearing two central and two lateral bristles and numerous (twelve to fourteen) short hairs. Post-scutellum brown. Pleurae rather lighter in colour than the dorsum. *Wings* grey. Anterior margin bearing only a single pale spot; this spot is situated on the apical side of the middle of the wing, just beyond the junction of the first and third veins with the costa. A second pale, oval, spot covers the anterior cross-vein and the basal portion of the first interspace, but does not reach to the costa. The other pale spots are situated as shown in the figure (Plate VIII, fig. 1); these are always less distinct than those on the anterior portion of the wing, and may be quite indistinct or absent. Venation as shown in the figure: first and third veins infuscated, forming two interspaces; anterior cross-vein oblique, pale coloured; fourth vein forking before the middle of the wing. Wings well clothed with decumbent hairs. Halteres, knobs dark brown, stalks paler, almost cream-coloured. *Legs* brown, dark knee-spots not conspicuous; tibiae with narrow, basal pale bands; tarsi of all the legs lighter in colour than the femora and tibiae. *Abdomen* dark brown in dried specimens. Spermathecae two, oval, unequal (in one specimen measuring 44μ by 39μ and 37μ by 30μ respectively), not very highly chitinised; duct chitinised only at its origin.

PUPA. Apparently almost indistinguishable from the pupa of *C. clarkei*. Average length of four females 2 mm. *Respiratory trumpets* similar to those of *C. clarkei*. Length about 0.2 mm.; stalks short. The trumpets bear three or four blunt knobs. *Cephalo-thorax*: spines on operculum less numerous posteriorly, as in *C. clarkei*. Anterior marginal tubercle bearing a long, stout, bristle; anterior dorsal, bearing a long hair and a short spine; dorso-lateral, bearing two moderately long hairs; ventro-lateral, bearing a long and a moderately long hair; ventro-

median, minute, bearing a minute hair. Dorsal tubercles: anterior, single, bearing a short, stout, spine; posterior, bearing a moderately long hair; lateral, bearing a long hair. Postero-dorsal tubercle bearing a rather stout, short, bristle. *Abdomen*: anal segment normal. Dorsal tubercles: antero-submarginal, each bearing a hair; postero-marginal, the outermost bearing a hair, the second a long, stout, spine, and the inner three minute spines. Lateral tubercles very large: antero-submarginal, bearing a long, strong, spine; postero-marginal, the middle one bearing a delicate seta, the other two long, strong, spines. Ventral tubercles: the middle one bearing a hair, the other two rather long spines.

LARVA. The larva is similar to that of *C. clarkei*. Length 3.6 mm. to 4.6 mm.; greatest breadth about 0.2 mm.

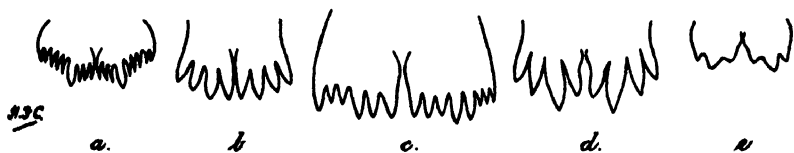


FIG. 21. Posterior margins of hypopharyngeal sclerites of larvae of: a—*C. accraensis*, sp.n.; b—*C. eriodendroni*, sp.n.; c—*C. nigripennis*, sp.n.; d—*C. clarkei*, sp.n.; e—*C. schultzei* (End.). ($\times 100$ circa.)

Head, length about 0.1 mm., greatest breadth about 0.08 mm. Eyes small, reniform. Bristles arranged similarly to those of *C. accraensis*. Mental plate cone-shaped, bearing four teeth on each side. Hypopharyngeal sclerite (fig. 21 b) with four teeth on each side posteriorly. Labrum with long papillae. Mandibles with a small tooth about the apical third. *Body*: bristles rather inconspicuous excepting those at the posterior end of the last segment, which are similar to those of *C. clarkei*.

HABITAT: Nsawam, Dodowah, and Oblogo, Gold Coast. Bred from larvae obtained from rot-holes in the stump of a silk-cotton tree (*Eriodendron anfractuosum*), a mango tree (*Mangifera* sp.), and another tree. All the specimens reared were females. January to March, 1920.

Culicoides nigripennis, sp. n.

Only two adult specimens of this midge, both females, were collected; the measurements of these in a dried condition were as follows:—

MEASUREMENTS.

| | | | | | | | | |
|--------------------------|-----|-----|-----|-----|-----|-----|-----|---------|
| Length of body | ... | ... | ... | ... | ... | ... | ... | 1.2 mm. |
| Length of wing | ... | ... | ... | ... | ... | ... | ... | 1.4 mm. |
| Greatest breadth of wing | ... | ... | ... | ... | ... | ... | ... | 0.5 mm. |

Head dark brown. Eyes narrowly separate, with the internal chitinous thickening very indistinct. Proboscis dark brown. Palpi dark brown, third segment moderately inflated in the middle. *Antennae* brown, the torus darker than the flagellum; segments four to ten, oval to sub-cylindrical, one and a half to two times as long as broad. *Thorax* dark brown, becoming dark grey posteriorly, and clothed with dark brown hairs; anteriorly there is an indication of a paler brown, broad, median stripe, and posteriorly, near the centre of the grey area, are two somewhat reniform dark spots. Scutellum dark brown, bearing two central and two lateral bristles and several (twelve) short dark hairs. Post-scutellum dark brown. Pleurae dark brown. *Wings* dark grey, almost uniformly coloured, but the anterior border is somewhat darker than the rest of the wing. Pale spots as shown in the figure (Plate VIII, fig. 2); a small spot covering the anterior cross-vein but not reaching the costa, another small spot upon the anterior margin immediately distal to the point at which the third vein joins the costa, and a slight indication of a light spot at the base of the wing between the first and fifth veins. Venation as shown in the figure. The wing surface is densely clothed with decumbent hairs. Halteres with brown knobs and paler coloured stalks. *Legs* almost uniformly dark brown, but with slight indications of pale, basal, bands on the tibiae; distal tarsal segments somewhat paler than the more proximal segments. *Abdomen* in dried specimens dark brown, with dark brown hairs; in fresh specimens there are minute pale spots, one on each side of the middle line, upon all the abdominal segments. Spermathecae (fig. 6c) two, thinly chitinated, considerably dilated, spherical sacs (diameter 65μ to 70μ) with the commencement of the duct chitinated for a relatively long distance (about 20μ).

PUPA. The following description is based on an examination of two pupal pelts from which females had hatched, and of eight dead pupae (two ♂ and six ♀) found in the material from a rot-hole in a mango tree. Length 2·1 mm. to 2·7 mm., average 2·5 mm. *Respiratory trumpets* rather short and broad; length about 0·2 mm. Stalk or pedicle moderately long. The trumpets are slightly broader at the base and apex than in the middle, and bear two or three very small knob-like processes. The main tracheal trunk terminates distally in a fan-like arrangement of nine short blunt processes. *Cephalo-thorax*: anterior marginal tubercle large, conical, bearing a stout bristle; anterior dorsal, large, conical, bearing a long hair and a very small spine; dorso-lateral large, bearing a long hair and a short spine; ventro-lateral bearing two hairs and, in some specimens, a third minute hair or spine; ventro-median very small, bearing a long, moderately stout hair and a short hair. Dorsal tubercles: anterior, single, bearing a rather long, stout, spine; posterior, bearing a long hair; lateral, bearing a long hair. Behind the posterior tubercle is another tubercle bearing a minute spine, and in front of the anterior tubercle is a rather large, unarmed, tubercle. Postero-dorsal tubercle bearing a long hair. *Abdomen*: anal segment with squamose spines arranged densely almost up to the tips of the processes, which are rather short and, in some specimens, black. Tubercles on the abdominal segments not strongly developed. Dorsal tubercles: antero-submarginal, the inner bearing a short spine and the outer a longer hair; postero-marginal, the outermost bearing a hair, the other four small or minute spines. Lateral tubercles not very large, but better developed than either the dorsal or ventral: antero-submarginal bearing a short spine; postero-marginal, the middle one bearing a hair, the other two short spines. Ventral tubercles: the outer one bearing a short spine, the middle one a hair, and the inner one a minute spine.

LARVA. In a sample of the contents of a rot-hole in a tree sent to us by Dr. F. H. Storey from Adawso, a town about thirty miles north of Accra, two *Culicoides* larvae were found together with a number of mosquito larvae (*Culiciomyia nebulosa*, Theo.). The sample had been sent through the post by a modification of Legendre's (1916) method of transportation, which we have found of considerable

service, and it is interesting to know that *Culicoides* larvae can be transported in this way. The two larvae were isolated separately; the one died, but the other pupated, and from it there emerged on the fourth day a female *C. nigripennis*. The pupal pelt was found floating in the water in the tube, but the larval pelt was not recovered. The following description is made from the larva which died, but which, from its large size, was probably also that of *C. nigripennis*.

Length 4.9 mm., greatest breadth 0.3 mm. *Head*, length 0.23 mm., greatest breadth 0.15 mm. Bristles normal. Mental plate semicircular with a median terminal, and four or five lateral teeth; these are very indistinct and, in the single specimen available, could not be exactly determined. Posterior margin of the hypopharyngeal sclerite as shown in fig. 21c. Palpi with two spines and a short rod at the apex. Antennae as in fig. 10, 4a. *Body*: bristles only moderately developed, the lateral ones relatively smaller than in *C. accraensis*, the arrangement apparently normal. Bristles at the posterior end of the anal segment normal.

HABITAT: Dodowah, Gold Coast: nine pupae found in débris collected from a rot-hole in a mango tree (*Mangifera* sp.), March, 1920; a female emerged from the single living pupa. Adawso, Gold Coast: one female reared from material obtained from a rot-hole in a tree, and sent to us by Dr. F. H. Storey; May, 1920.

C. nigripennis is the largest species of *Culicoides* yet obtained in the Gold Coast; its size, dark coloration and densely hairy wings enable it to be identified with ease.

Culicoides similis, sp. n.

| MEASUREMENTS. | | | | | | Female. | Male. |
|--------------------------|-----|-----|-----|-----|-----|---------|---------|
| Length of body | ... | ... | ... | ... | ... | 1.3 mm. | 1.0 mm. |
| Length of wing | ... | ... | ... | ... | ... | 1.1 mm. | 0.9 mm. |
| Greatest breadth of wing | ... | ... | ... | ... | ... | 0.4 mm. | 0.3 mm. |

Head brown; occiput dark brown with a few dark brown hairs. Eyes separate in both sexes; in the female the internal chitinous thickening is poorly developed. Proboscis brown. Palpi brown; the third segment considerably swollen and containing a large pit. *Antennae*: torus dark brown, flagellum segments brown bearing

brown hairs. In the female the seven basal segments (four to ten) short, sub-spherical to broadly ovoid, very slightly longer than broad; in the male the last three segments sub-equal. *Thorax* dark brown with pale brownish-grey markings as shown in fig. 22*f*. Dorsum sparsely clothed with brown hairs. Scutellum pale greyish- or yellowish-brown, almost orange-coloured in fresh specimens, with

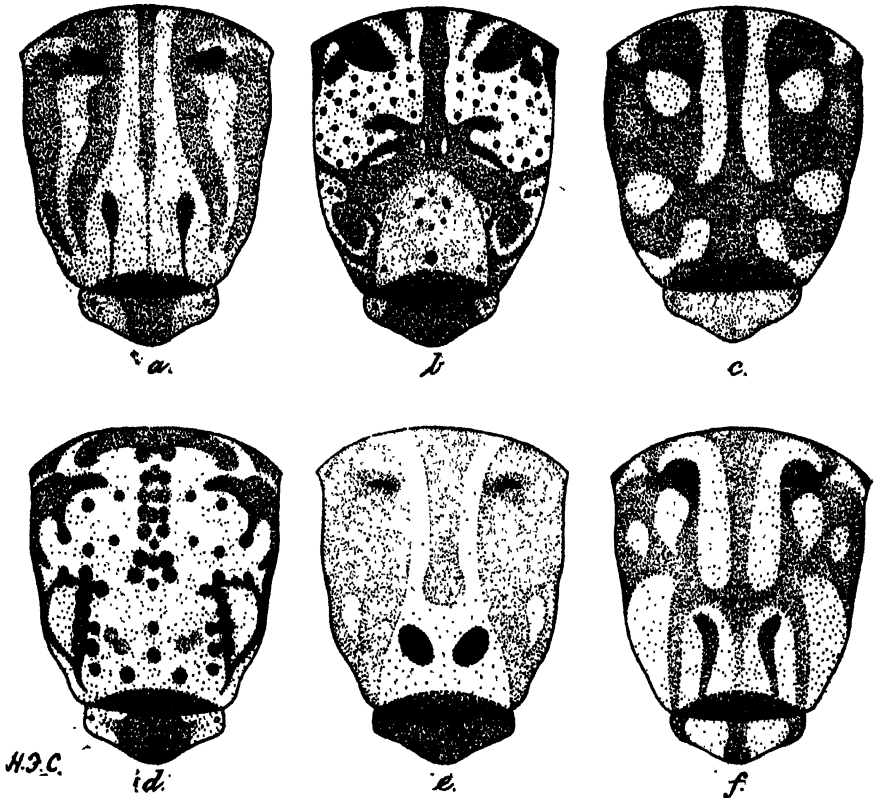


FIG. 22. Thoracic ornamentation of: a—*C. clarkei*, sp. n.; b—*C. punctatorax*, sp. n.; c—*C. smorastipennis*, sp. n.; d—*C. schultzei* (End.); e—*C. accraensis*, sp. n.; f—*C. similis*, sp. n. (semi-diagrammatic.)

a dark median band and dark patches at each side, bearing, in both sexes, two central and two lateral bristles and a few short hairs. Post-scutellum dark brown. Pleurae coloured in a similar manner to the dorsum. *Wings* grey; anterior border rather darker than the rest of the wing and showing successively a light area at the base,

a diffuse dark area, a light-coloured spot covering the basal two-thirds of the proximal interspace and the anterior portion of the cross-vein, a very dark small rectangular spot in the middle of the anterior border at the junction of the first and third veins with the costa, a light coloured spot often divided into two in a more or less transverse direction, a large dark spot less dense than the dark spot at the middle of the anterior margin, and finally a triangular light-coloured spot just before the apex of the wing. These markings and the other pale spots on the wing are shown in the figure (Plate VIII, fig. 6); the three or four pale spots in the neighbourhood of the bifurcation of the fifth vein are of importance in differentiating this species from the following one (*C. citroneus*). Venation as shown in the figure; first and third veins not completely united, but forming two cells; anterior cross-vein oblique, pale at its anterior end; fourth vein forking about the middle of the wing. Wing surface sparsely clothed with decumbent hairs, some of which, however, extend almost to the base of the wing between the fourth and fifth veins. Halteres yellowish, the distal portion of the stem narrowly grey. *Legs* brown, with dark knee-spots; femora with indistinct paler areas near the apex; tibiae with pale basal bands, and some indication of apical pale bands also on the middle and hind legs; tarsal segments rather lighter in colour than the femora and tibiae. *Abdomen* dark brown in dried specimens, clothed with short, dark, hairs. Spermathecae two, not very highly chitinised; ovoid or pyriform (length about 50μ , breadth about 42μ); chitinised portion of the duct long, about 12μ , and slightly tapering.

HYPOPYGIUM (fig. 23). *Ninth segment*: sternite with a shallow depression; tergite rather narrow posteriorly, and sparsely clothed with hairs of only moderate length; posterior margin not notched, ending on each side in a rather small finger-like process. *Forceps* of the usual form; side-piece with two sub-dorsal processes at its proximal end, the more posterior of which bears basally a short, blunt, backwardly directed structure. *Harpes* stout, branched appendages closely resembling those of *C. accraensis* (c.f. fig. 20 a and b); they differ from those of the latter species chiefly in the outline of the inner margin of the proximal portion being more sinuous, so that a more pronounced bulge is present above the middle, and in

the shorter branch being more slender and pointed; the number of serrations on the posterior edge of the blade varies from six to nine. *Aedoeagus*: stem not highly chitinised, very long, and forming (as seen in a lateral view) almost a semicircle with its concave surface ventral; termination filiform. Limbs highly chitinised, narrow, with basal extremities slightly everted; they form a low, broad, arch with a rounded apex. Ventral wall chitinised; membrane between the arch of the aedoeagus and the ninth segment without spines.

HABITAT: Accra, Gold Coast: collected in the evening upon the windows of the laboratory. Oblogo, Gold Coast: reared from material taken from a canoe in the river Densu. January to April, 1920.

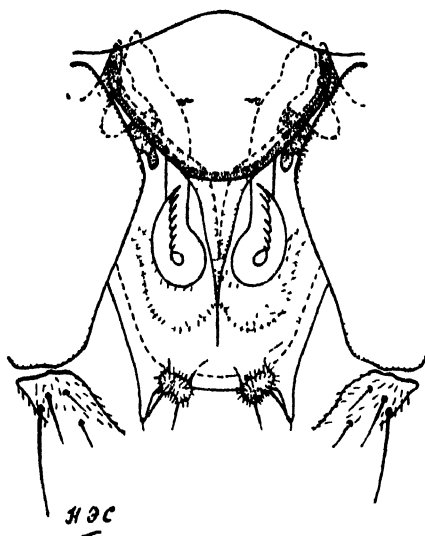


FIG. 23. *C. similis*, sp.n., male hypopygium, ventral view. ($\times 400$.)

The wing markings of this species resemble those of the following species (*C. citroneus*), but the two insects can be distinguished by the arrangement of the pale spots in the neighbourhood of the bifurcation of the fifth vein. The genitalia of the males, however, are quite distinct, the structure of the harpes apparently indicating relationship with *C. accraensis*.

Culicoides citroneus, sp. n.

| MEASUREMENTS. | | | | | | Female. | Male. |
|--------------------------|-----|-----|-----|-----|-----|---------|---------|
| Length of body | ... | ... | ... | ... | ... | 1.2 mm. | 0.8 mm. |
| Length of wing | ... | ... | ... | ... | ... | 1.0 mm. | 0.7 mm. |
| Greatest breadth of wing | ... | ... | ... | ... | ... | 0.4 mm. | 0.3 mm. |

Head brown, occiput dark brown with dark hairs. Eyes in the female very narrowly separate; internal chitinous thickening distinct. Proboscis and palpi brown, the third segment considerably swollen. *Antennae*: torus brown, flagellum segments lighter brown bearing brownish hairs. Seven basal segments (four to ten) in the female elongate, from nearly two to almost two and a half times as long as broad. In the male the thirteenth segment longer than either the fourteenth or fifteenth. *Thorax*: ornamentation similar to that of the preceding species (*C. similis*), chiefly differing as follows: the pale areas rather darker brown, less distinct; the admedian pale stripes not divided by a transverse dark band near the centre, *i.e.*, the pale areas on the anterior part are continuous with those on the posterior part of the thorax; the large admedian pale spots broadly fused with the admedian stripes; the conspicuous black oval spots on the posterior part not prolonged to the posterior margin by dark stripes. Dorsum sparsely clothed with brown hairs. Scutellum also marked in a similar manner to that of the preceding species, but less distinctly; bearing two central and two lateral bristles, and, in the female, a few short hairs also. Post-scutellum dark brown. Pleurae with colouration similar to that of the dorsum. *Wings* with markings similar to those of the preceding species (*C. similis*), the chief differences being: the pale spot situated near the middle of the anterior border covering the proximal half only of the first cell and completely enveloping the cross-vein (see Plate VIII, fig. 5), and the presence of a small pale area in the angle of the fork of the fifth vein. Venation also differing slightly: the petiole of the cell enclosed by the rami of the fourth vein distinctly longer, and the cell itself less attenuated basally, than in the preceding species. Wings sparsely clothed with decumbent hairs, but with a few (between veins four and five) extending beyond the anterior cross-vein. Halteres with bright lemon-yellow coloured knobs. *Legs* brown; fore legs with con-

spicuous dark knee-spots, above and below which are narrow pale bands; middle legs with pale knee-spots, most marked on the tibial side; hind legs with a conspicuous narrow, pale, band at the base of the tibiae. *Abdomen* dark brown in dried specimens, clothed with short, dark, hairs. *Spermathecae* two, rounded or oval, moderately well chitinised, diameter about 50μ ; practically no part of the commencement of the duct is chitinised.

HYPOPYGIUM (fig. 24). *Ninth segment* of the usual form; posterior margin of the tergite notched in the middle and ending, on each side, in a relatively long, narrow, finger-like process. *Forceps*: side-pieces normal; small hairs extending about two-thirds the length of the claspers. *Harpes* (fig. 20c) simple, tapering to a

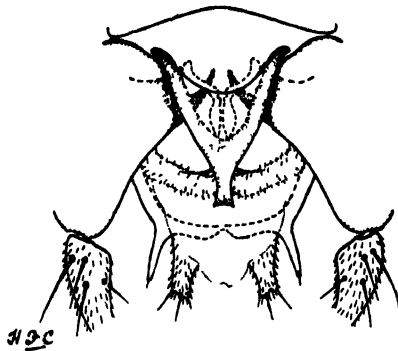


FIG. 24. *C. citreus*, sp.n., male hypopygium, ventral view. ($\times 400$.)

thread-like termination, root-like basal portion highly chitinised and almost at a right angle to the terminal portion; terminal portion slightly sinuous, attenuated before the middle and with the end bent sharply ventrally. *Aedoeagus* Y-shaped, gradually tapering to a distal, rather broad, gutter-like process with a blunt termination; in ventral view, their extremities apparently turned slightly outwards; the arch formed by the limbs relatively narrow and pointed. Ventral wall chitinised, the membrane connecting it with the ninth sternite not spiculated.

HABITAT: Accra, Gold Coast. Collected in the evening upon the windows of the laboratory from December, 1919, to April, 1920; never abundant, and very uncommon in February.

Culicoides austeni, sp. n.

Culicoides milnei, Aust. (pro parte). Ann. Mag. Nat. Hist. Ser. 8, Vol. III, p. 283, 1909; Bull. Ent. Res., Vol. III, p. 100, 1912.

In 1912, Austen noted that specimens of *C. milnei* from the Anglo-Egyptian Sudan and Southern Nigeria differed from the type form from Nairobi 'in being considerably smaller, and in having the two distal light costal spots on the wings much closer together' and suggested that in these places it was apparently represented by a local race. A careful comparison of the type and paratypes of *C. milnei*, Aust., with the Southern Nigerian (Yaba) specimens preserved in the British Museum collections leads us to believe that the so-called small race of *C. milnei*—which occurs in the Congo and Uganda as well as in British West Africa—is specifically distinct, and we therefore propose for it the name *C. austeni*.

Apart from the distinguishing characters mentioned by Austen, this species differs from the true *C. milnei* in the more distal of the two small cells or interspaces, enclosed by the first and third veins, being distinctly narrower, and in the decumbent wing-hairs extending further towards the base of the wing between the upper branches of the fourth and fifth veins. In *C. austeni* the hairs in this area extend almost to the middle of the wing, whereas in *C. milnei* they reach to about the apical third only. Further, a microscopic preparation of a female received from Nairobi shows that the eyes are distinctly, though narrowly, separate above, while in females of *C. austeni* they are contiguous. This character cannot, as a rule, be accurately determined in dry specimens, and in the type and paratypes could not be satisfactorily observed; but as the Nairobi example in our possession agrees in all other details with the type of *C. milnei* we feel justified in regarding it as Austen's species, and in giving as an additional and important distinguishing feature between females of *C. milnei* and *C. austeni* the separation or contiguity of the eyes.

We have collected at Accra numerous specimens of *Culicoides austeni*, and have been fortunate in obtaining both males and females. The male has been previously collected, and Austen (1912) refers to one taken at Yaba near Lagos. The males collected by us were similar to the females, but of slighter build and with

the eyes narrowly separate. The average measurements of three specimens after immersion in pure carbolic were as follows:—Length of body, 1·2 mm.; length of wing, 1·1 mm.; greatest breadth of wing, 0·3 mm. In the female the eyes are broadly contiguous above; antennal segments four to ten are oval to sub-cylindrical, from one and a half to two and a quarter times as long as broad. Palpi as shown in fig. 3 *a*. Spermathecae (fig. 6 *d*) two in number, dark coloured, highly chitinised, sub-spherical—the diameter about 33μ ; the duct narrow, chitinised for a very short distance (about 3μ) only. In both sexes the scutellum bears two central and two lateral bristles and, in the female, additional short hairs.

HYPOPYGIUM (fig. 25). *Ninth segment*: ventral depression in the sternite of moderate depth; tergite of about the usual length,

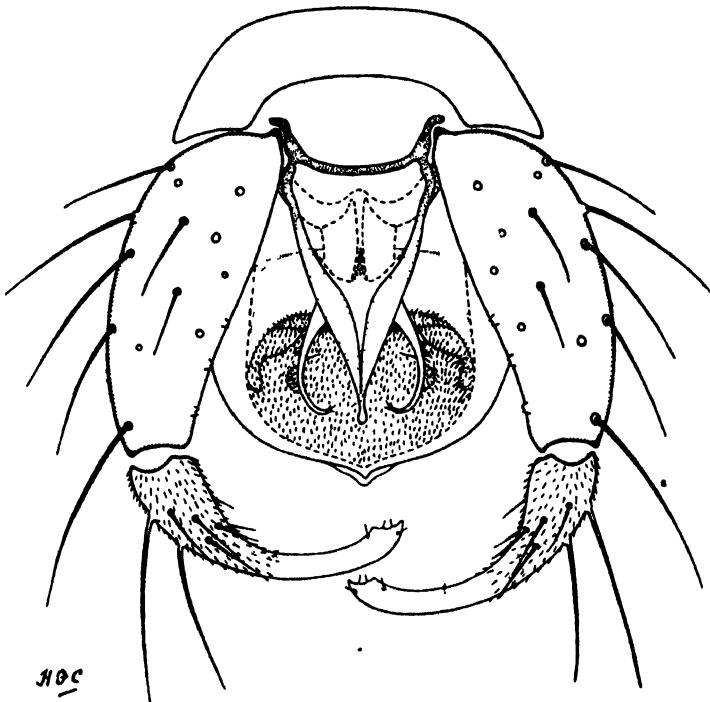


FIG. 25. *C. austeni*, sp.n., male hypopygium, ventral view. ($\times 400$.)

almost as broad posteriorly as at the base, sparsely clothed with strong hairs which are most numerous over the posterior third; posterior margin slightly notched in the middle, with a line extending from the notch for a short distance in an anterior

direction; posterior margin without lateral processes, but with a small triangular projection in the middle behind the notch. *Forceps*: side-pieces highly chitinated and hairy; end of clasper only slightly depressed. *Harpes* (fig. 20 *l*) unbranched; the proximal or basal portion directed laterally, approximately at a right angle to the rest of the appendage, highly chitinated, with the external extremity bent anteriorly; the distal portion long, not very highly chitinated, gradually tapering to a filiform end which bears a few minute hairs; general direction ventral, but considerable twisting, especially distally, occurs. *Aedoeagus* V-shaped rather than Y-shaped, concave ventrally; distal portion long, not very highly chitinated, concave ventrally, and tapering to end in a small knob; limbs more highly chitinated, especially at their bases, forming a somewhat narrow, pointed, arch, the acute apical angle of which is occupied by a small tooth-like projection of the dorsal wall; ventral wall chitinated, limited anteriorly by a highly chitinated transverse band, beyond which the limbs project slightly. The membrane connecting the ventral wall with the abdomen appears to be without spicules.

HABITAT. Accra: collected in the evening upon the windows of the laboratory, January to April, 1920; and one specimen taken in a bungalow in March. Sekondi: one specimen collected by Dr. W. G. Watt, April, 1920.

Culicoides grahami, Aust.

Culicoides grahamii, Aust. Ann. Mag. Nat. Hist., Ser. 8, Vol. III, p. 280, 1909; Illustrations of African Blood-sucking Flies, p. 7, Pl. I, fig. 3, 1909.

Culicoides habereri, Becker. Jahreshefte des Vereins für vaterland. Naturkunde in Württemberg, Jahrg., 1909, p. 289, Taf. VIII, Bd. IX 1909.

? *Oecacta hostilissima*, Pittaluga. Centr. Bakt. Abt. I, Bd. 59. Orig. p. 69, 1911.

Numerous examples of this species were collected both at the Accra laboratory and at Tafo and villages in the Koforidua district, but in spite of its apparent abundance no breeding-places were located.* Males were obtained only on the laboratory windows.

MEASUREMENTS.

| | | | | | Female. | Male. |
|--------------------------|-----|-----|-----|-----|----------------|---------|
| Length of body | ... | ... | ... | ... | 0.9 to 1.2 mm. | 1.0 mm. |
| Length of wing | ... | ... | ... | ... | 1.0 mm. | 0.9 mm. |
| Greatest breadth of wing | ... | ... | ... | ... | 0.4 mm. | 0.3 mm. |

* Since the above was written, larvae of *C. grahami* have been found in rotting material at the bases of banana plants.

The eyes of the female, as described by Becker, are contiguous dorsally for a somewhat variable distance; in the male they are narrowly separate, almost contiguous at the vertex. The third palpal segment is but slightly swollen. The antennae of the female are of moderate length, the basal segments (four to ten) varying from about one and a half to two times as long as broad, the last antennal segment of the male is slightly longer than either of the two preceding segments. The scutellum of the female bears three bristles, one central and two lateral, and is devoid of short hairs; that of the male bears a central bristle only (c.f. fig. 5, *a* and *b*). In our specimens the pale spots on the wings are more numerous than shown in Austen's figure, but the additional ones are ill-defined. The latter are situated along the middle line of the wing, notably at the base, and on each side of the termination of the lower ramus of the fourth vein. The decumbent hairs are not entirely confined to the upper portion of the distal extremity of the wing, and are present, though extremely scanty, along the apical and posterior margins. In the male the hairs are arranged as in the female, but are even less numerous. The spermathecae are dark and highly chitinated, variable in number and size; two or, occasionally, three, are present, and although usually almost spherical in shape (diameter about 40μ) are sometimes oval. The duct is chitinated at its commencement for but a short distance.

HYPOPYGIUM (figs. 26 and 27). *Ninth segment*: sternite with the excavation moderately deep; tergite relatively short, the posterior margin notched centrally, without finger-like processes but extended into a short, rounded, flange on each side of the middle line. Apical, lobe-like, processes moderately well-developed. *Forceps*: basal half of clasper covered with minute hairs and bearing also a few relatively strong hairs; distal portion with four or five minute hairs, the apex pointed. *Harpes*: distal portion long, narrow, unbranched, rather strongly curved and bent ventrally at the apex, which is filiform and bears a few minute hairs; proximal portion rather more highly chitinated than the rest of the harpe, directed slightly dorsally. *Aedoeagus* of normal form (Y-shaped) with the distal extremity bent ventrally. The distal portion is rather short and narrow, not very highly chitinated, with a bluntly rounded end; the limbs are more highly chitinated, especially at

their proximal, everted ends, and form a narrow arch over the excavation in the ninth sternite; the apex of the arch is occupied by a chitinised, pointed, spine-like prolongation of the dorsal wall. The ventral wall is distinctly chitinous and the membrane connecting it with the ninth sternite bears a few minute spicules.

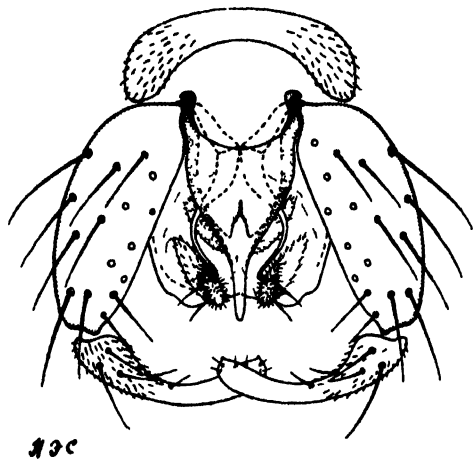


FIG. 26 *C. grabami*, Aust, male hypopygium, ventral view (spicules on anterior portion of membrane, connecting ventral wall of aedoeagus to ninth sternite, omitted) ($\times 400$)

HABITAT · Accra, Gold Coast, both males and females collected in the evening on the windows of the laboratory from December, 1919, to April, 1920, but very uncommon in February. Females also collected at Tafo, and villages in the Koforidua district by Dr. F. H. Storey.

Culicoides pallidipennis, sp. n.

MEASUREMENTS.

| | | | | | Female. | Male. |
|--------------------------|-----|-----|-----|-----|----------------------|---------|
| Length of body | ... | ... | ... | ... | 1.3 mm. | 1.1 mm. |
| Length of wing | ... | ... | ... | ... | 1.1 mm. ¹ | 0.9 mm. |
| Greatest breadth of wing | ... | ... | ... | ... | 0.4 mm. | 0.3 mm. |

Head dark brown. Eyes contiguous in the female, narrowly separate in the male. Proboscis and palpi dark brown, the latter with the third segment slender, scarcely at all dilated. *Antennae* brown, the first two segments darker than the rest. Segments four to ten in the female, oval to sub-cylindrical, from once and

a half to twice as long as broad; thirteenth segment in the male, slightly longer than the terminal segment. *Thorax* rather dark greyish-brown, clothed with pale brown hairs; the anterior pit and two elliptical areas near the centre of the posterior depression darker, almost black. Scutellum in both sexes dark greyish-brown, with bristles as in *C. grahamsi*. Post-scutellum dark brown. Pleurae dark brown. *Wings* with very extensive pale markings as shown in Plate VIII, fig. 3. Decumbent hairs of the wings scanty and almost confined to the apical and posterior marginal areas; they are most numerous in the distal anterior portion above the upper branch of the fourth vein. Halteres with cream-coloured knobs and slightly infuscated stems. *Legs* dark brown, the tarsi slightly paler; knee-spots dark, the tibiae with indications of narrow

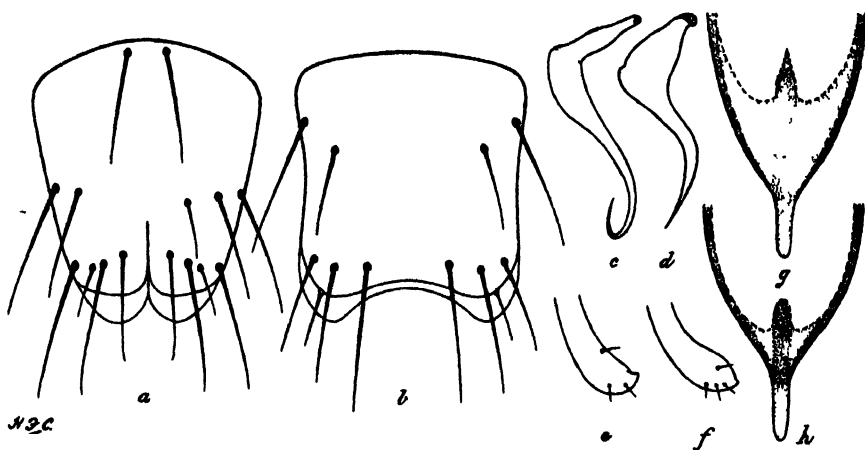


FIG. 27. Hypopygial details of the males of *C. grahamsi*, Aust (a, c, e and g), and *C. pallidispennis*, sp. n. (b, d, f and h) a and b—ninth tergites; c and d—harpes; e and f—extremities of claspers; g and h—aedeagus. (a and b $\times 360$, c—b $\times 490$.)

pale bands at the base. *Abdomen* dark brown, almost black distally, clothed with dark brown hairs. Spermathecae two, dark and highly chitinised, sub-spherical, about 50μ by 45μ ; a very short portion of the duct is chitinised.

HYPOPYGIUM: strikingly similar in structure to that of *C. grahamsi*. The chief differential points are shown in fig. 27. The ninth tergite is less hairy, relatively broader posteriorly, and not partially cleft in the middle line of the dorsal surface. The distal extremity of the clasper is rather broader and more rounded. The

harpes are shorter and stouter with the basal portions markedly broader. The dorsal wall is less extensive and its spine-like anterior continuation, lying between the limbs of the aedoeagus, is shorter and blunter.

HABITAT: Accra, Gold Coast; collected upon the windows of the laboratory in the evening from December, 1919, to April, 1920, but never abundant, and very uncommon in February.

The close relationship existing between this species and *C. grahami*, Aust., is shown in several ways, notably in the structure of the male hypopygium and the scantiness of the decumbent wing-hairs. The great development of the pale markings of the wings of *C. pallidipennis*, however, will enable the species to be separated with ease. In regard to the last-named character, *C. pallidipennis* resembles *C. brucei*, Aust. The latter species, however, is more robust in build, and possesses sepia-coloured halteres and more numerous decumbent wing-hairs. The wing markings, moreover, are by no means identical, the chief differences being the absence of the small grey spot in the large pale area situated near the tip of the wing above the upper branch of the fourth vein, the more proximal position of the dark transverse band between the branches of the fourth vein, and the absence of the small but sharply defined grey spot in the centre of the cell enclosed by the branches of the fifth vein.

SYNOPTIC TABLES

The chief distinguishing characters of the species of *Culicoides* now known to occur in the Gold Coast are summarised in the following tables:—

FEMALES.

| | | | | |
|----|---|-----|---|-------------------------------------|
| 1. | Wings, uniformly coloured—without spots | ... | <i>inornatipennis</i> , sp. n. (p. 227) | |
| | Wings, with distinct pale spots or areas | ... | ... | 2 |
| 2. | Thorax, entirely ochraceous | ... | ... | <i>fulvithorax</i> (Aust.) (p. 230) |
| | Thorax, grey or brown | ... | ... | 3 |
| 3. | Thorax, grey or with large grey areas conspicuously spotted dark brown or black | ... | ... | 4 |
| | Thorax, almost uniformly coloured or otherwise adorned | ... | ... | 5 |

4. First and third longitudinal veins, separate distally, forming two distinct cells; apical anterior portion of wing with one small pale spot at end of costa *punctithorax*, sp. n. (p. 235)
 First and third longitudinal veins, entirely fused; apical anterior portion of wing with (usually) three or four pale spots, or (if united) with a large semicircular pale area *schulzei* (End). (p. 231)
5. Decumbent wing-hairs, dense; numerous in the basal portion of the wing between the fourth and fifth veins (i.e. from the origins of the veins as far as the anterior cross-vein) 6
 Decumbent wing-hairs, sparse or scanty; at most a single row of hairs in the basal portion of the wing between the fourth and fifth veins 12
6. Wings, with a pale spot (not connected with the pale spots at the cross-vein and end of costa) immediately below the junction of the two small anterior cells formed by the first and third veins 7
 Wings, without such spot 8
7. Wings, with a small circular or oval pale spot just beyond and below the conspicuous spot at the end of the costa; spermatheca single, strongly chitinated, oval *praetermissus*,* sp. n. (p. 240)
 Wings, without such a spot; spermatheca single, very delicately chitinated anteriorly, peg-top shaped *distinctipennis*, Aust. (p. 238)
8. Wings, with a small pale spot distally above the end of the upper branch of the fourth vein, and with distinct spots near the distal and posterior margins 9
 Wings, without a pale spot distally above upper branch of fourth vein; pale spots near distal and posterior margins absent or ill-defined 11
9. Anterior cross-vein and base of proximal interspace, formed by first and third veins, dark—not enveloped by the pale spot
 *accraensis*, sp. n. (p. 241)
 Anterior cross-vein and base of proximal interspace enveloped by pale spot 10
10. Wings, with four sharply-defined pale spots forming an interrupted transverse band in the apical third, extending from the pale spot at the end of the costa to the pale spot in the distal angle of the fork of the fifth vein *neavei*, Aust. (p. 245)
 Wing spots not forming such a band *clarkiei*, sp. n. (p. 246)
11. Wings, usually with some ill-defined pale spots near distal and posterior margins; spermathecae normal, oval, the duct scarcely, if at all, chitinated; smaller species (wing length 1.1 mm.)
 *eriodendroni*, sp. n. (p. 250)
 Wings, without any indication of distal or posterior pale spots; spermatheca very large, spherical, the duct chitinated for a relatively long distance; larger species (wing length 1.4 mm.)
 *nigripennis*, sp. n. (p. 253)

* The character of the spermatheca is based on the examination of a female of what appears to be this species sent by Mr. G. A. H. Bedford from Pretoria; the wing markings agree with those of the male from West Africa.

| | | |
|-----|---|--|
| 12. | Wings, with pale spot at the end of the costa covering at most the extreme tip of the costa and third vein | 13 |
| | Wings, with pale spot or area covering at least one third of the distal interspace between the first and third veins | 15 |
| 13. | Fork of fifth vein, with two pale spots—one small, situated immediately below the point of bifurcation | <i>citronaeus</i> , sp. n. (p. 259) |
| | Fork of fifth vein, with one large pale spot | 14 |
| 14. | Decumbent wing-hairs sparse, but between the fourth and fifth veins reaching as far as the cross-vein and sometimes continued beyond it as a single row; a clearly defined pale spot immediately above the bifurcation of the fifth vein | <i>similis</i> , sp. n. (p. 255) |
| | Decumbent wing-hairs scanty, not reaching beyond the middle of the wing between the fourth and fifth veins; no pale spot above the bifurcation of the fifth vein | <i>confusus</i> , sp. n. (p. 250) |
| 15. | Decumbent wing-hairs sparse, those between the branches of the fourth vein reaching to about the middle of the wing | |
| | | <i>austeni</i> , sp. n. (p. 261) |
| | Decumbent wing-hairs, below the upper branch of the fourth vein extremely scanty, almost confined to the margins | 16 |
| 16. | Wings, appearing pale with dark areas | <i>pallidipennis</i> , sp. n. (p. 265) |
| | Wings, appearing dark with pale spots | <i>grahami</i> , Aust. (p. 263) |

The characters given in the above table will, for the most part, apply to the males also. In one important respect, however, namely, the density of the decumbent wing-hairs, the males differ considerably from the females; these hairs are always less numerous in the males, and although their density varies in different species, the differences are much less easily appreciated than in the females. A table of the males based on the characters of the hypopygium is, therefore, appended.

MALES.

| | | |
|----|---|---|
| 1. | Posterior margin of ninth tergite, with conspicuous lateral finger-like processes | 2 |
| | Posterior margin of ninth tergite, without finger-like processes | 11 |
| 2. | Membrane, connecting ventral wall of aedoeagus with ninth tergite studded with spicules | 3 |
| | Membrane, connecting ventral wall with tergite devoid of spicules | 5 |
| 3. | Distal portion of aedoeagus straight, broad and gutter-like; harpes long and tapering, sharply bent near the middle | |
| | | <i>schultzei</i> (End.) (p. 231) |
| | Distal portion of aedoeagus narrow, expanding into a mushroom-like apex; harpes short with the tips only bent | 4 |
| 4. | Extreme apex of harpes only bent; membrane connecting ventral wall of aedoeagus with ninth sternite completely studded with spicules | <i>distinctipennis</i> , Aust. (p. 238) |
| | Apical third of harpes bent; membrane spiculated on anterior two-thirds only | <i>praeternissus</i> , sp. n. (p. 24c) |

5. Harpes, large and broad, with serrated blade-like distal portions ... 6
 Harpes, tapering to a pointed apex, the distal portion usually filiform ... 7
6. Distal portion of aedoeagus straight, broad, and gutter-like ...
 *accraensis*, sp. n. (p. 241)
 Distal portion of aedoeagus tapering, the apical third filiform ...
 *similis*, sp. n. (p. 255)
7. Finger-like processes of ninth tergite, 8-9 times as long as the width
 in the middle *punctithorax*, sp. n. (p. 235)
 Finger-like processes of ninth tergite, at most 7 times the width in
 the middle (except in *C. inornatipennis* not more than 6×1) ... 8
8. Distal portion of aedoeagus, slender, slightly tapering; ventral wall
 not at all or very delicately chitinised, appearing continuous
 with the membrane connecting it with the ninth sternite ...
 *clerkei*, sp. n. (p. 246)
 Distal portion of aedoeagus, stout and blunt; ventral wall distinctly
 chitinised, the line of demarcation between it and the connecting
 membrane conspicuous 9
9. Finger-like processes of ninth tergite, short and stout (about 3×1)
 *neavei*, Aust. (p. 245)
 Finger-like processes, $6-7 \times 1$ 10
10. Filiform distal extremity of harpes with a single sharp ventral bend
 near the middle *citronaeus*, sp. n. (p. 259)
 Filiform distal extremity of harpes twisted ventrally, corkscrew-like
 *inornatipennis*, sp. n. (p. 227)
11. Posterior margin of ninth tergite, with a triangular projection in
 the middle; ventral wall of aedoeagus bounded anteriorly by
 a strong chitinous transverse bar *austeni*, sp. n. (p. 261)
 Posterior margin of ninth tergite, without a central projection, but
 with broadly rounded flanges on each side of the middle line:
 ventral wall of aedoeagus without chitinous bar 12
12. Ninth tergite, tapering posteriorly and partially cleft in the middle
 dorsal line *grahami*, Aust. (p. 263)
 Ninth tergite, almost rectangular, without a dorsal cleft
 *pallidipennis*, sp. n. (p. 265)

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EXPLANATION OF PLATES

PLATE VII

- Fig. 1. *C. inornatipennis*, sp. nov., female wing.
- Fig. 2. *C. schultzei* (End.), female wing; (a) portion of anterior proximal area of male wing.
- Fig. 3. *C. praetermissus*, sp. nov., male wing.
- Fig. 4. *C. punctithorax*, sp. nov., female wing.
- Fig. 5. *C. accraensis*, sp. nov., female wing.
- Fig. 6. *C. clarkei*, sp. nov., female wing.

NOTE.—The magnification in all the figures is approximately
× 90.

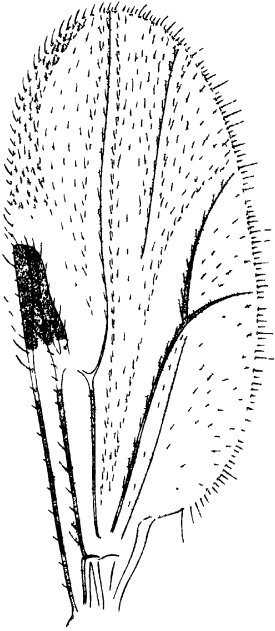


FIG. 4

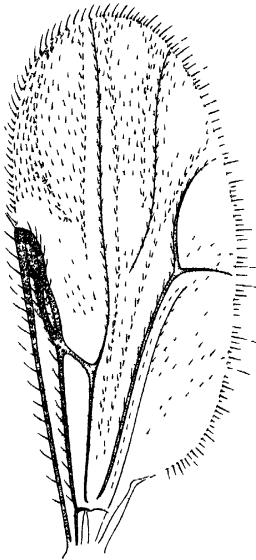


FIG. 5

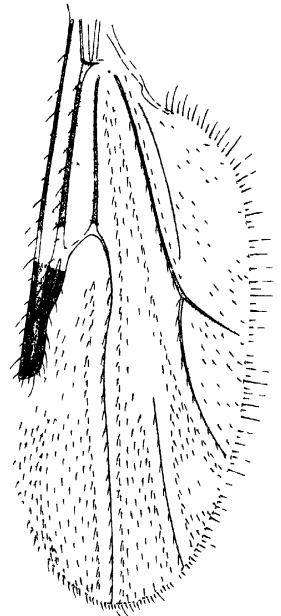


FIG. 6

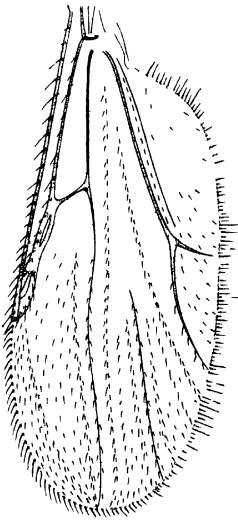


FIG. 1

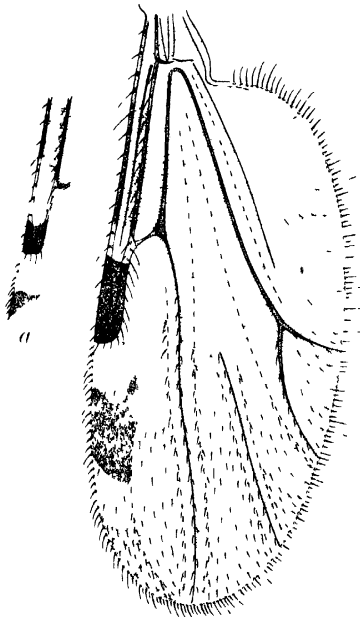


FIG. 2

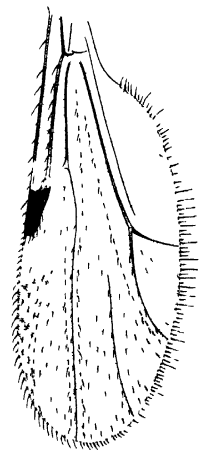


FIG. 3

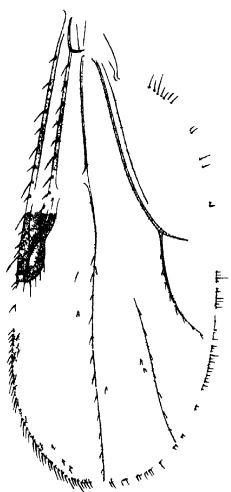
PLATE VIII

- Fig. 1. *C. eriodendroni*, sp. nov., female wing.
Fig. 2. *C. nigripennis*, sp. nov., female wing.
Fig. 3. *C. pallidipennis*, sp. nov., female wing (small specimen)
Fig. 4. *C. confusus*, sp. nov., female wing.
Fig. 5. *C. citroneus*, sp. nov., female wing.
Fig. 6. *C. similis*, sp. nov., female wing.

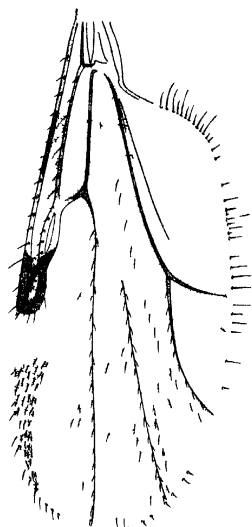
NOTE.—The magnification in all the figures is approximately
× 90.



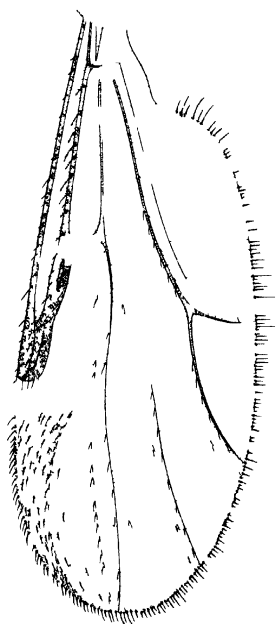
I 4



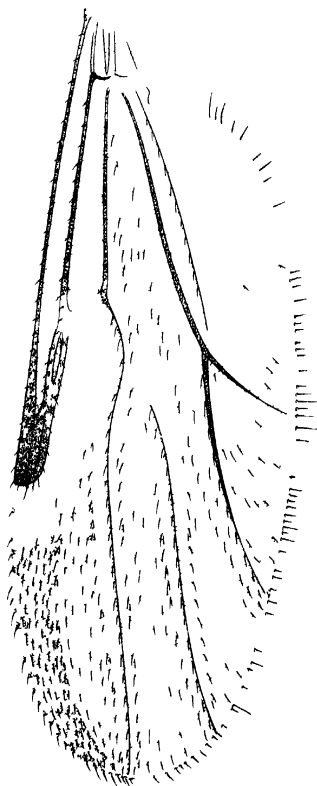
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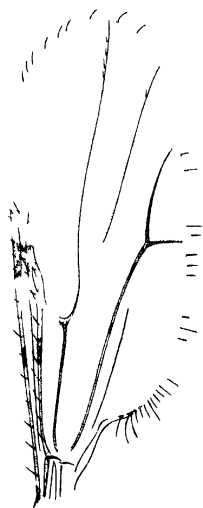
I 6



I 7



I 8



I 9

FURTHER EXPERIMENTS WITH *ANOPHELES PLUMBEUS*, STEPHENS; ITS INFECTION WITH *P. FALCIPARUM* IN ENGLAND; ALSO NOTES ON THE APPARATUS AND TECHNIQUE EMPLOYED

BY
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AND
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(Received for publication 28 September, 1920)

PLATE IX

In a recent paper (1920) we recorded the results obtained from dissections of laboratory-bred females of *Anopheles plumbeus*, which had fed on patients infected with *Plasmodium vivax*. The present paper gives the results of additional experiments with females fed on patients infected with *P. vivax* (mosquitoes kept at room temperature) and of feeding examples of this mosquito, bred in the same way, on a patient whose blood contained *Plasmodium falciparum* (mosquitoes kept at 28° C.).

EXPERIMENTAL

This work was carried out at Liverpool during the months of May and June, 1920.

I. *Experiments with laboratory-bred A. PLUMBEUS and P. VIVAX at room temperature.*

The mosquitoes used in these experiments were kept in a cupboard in the laboratory. Any that died after the infected feed were, if possible, dissected and examined in 0·85 per cent. sodium chloride. Examination of the peripheral blood of the two cases on

which these experiments were performed (B and C on the first case, D on the second) showed numerous parasites in both, but the process of exflagellation was observed in the second only.

TABLE I.

Showing the results of feeding laboratory-bred *A. plumbeus* on patients infected with *P. vivax*.

| Exp. | Date Fed | NUMBER | | | | | Temperature used Min. - Max. | Remarks |
|------|----------|--------|----------------|----------|-----|----------------|---------------------------------|---|
| | | Fed | Dis- sected | Infected | Gut | Sal. Glands | | |
| B | 8.5.20 | 5 | 5 | ... | ... | ... | 13° C.-22° C. | Females killed from 10th to 22nd day after infected meal. |
| C | 8.5.20 | 5 | 5 | .. | ... | ... | 13° C.-22° C. | Females killed (one died 19.5.20) 14th to 18th day after infected meal. |
| D | 14.5.20 | 2 | 2 | ... | ... | ... | 13° C.-22° C. | Females killed on 18th and 19th day after infected meal. |

II. *Experiments with laboratory-bred A. PLUMBEUS and P. FALCIPARUM.*

The mosquitoes used in this experiment were kept in an incubator at 28° C. The blood of the patient upon which the mosquitoes fed was examined immediately before feeding; gametes were present, but the process of ex-flagellation was not observed.

TABLE II.

Showing the results of feeding laboratory-bred *A. plumbeus* on a patient infected with *P. falciparum*.

| Exp. | Date Fed | NUMBER | | | | | Temperature used | Remarks |
|------|----------|--------|----------------|----------|-----|----------------|------------------|--|
| | | Fed | Dis- sected | Infected | Gut | Sal. Glands | | |
| A | 12.6.20 | 12 | 11 | 1 | 1 | ... | 28° C. | Ten had died by the 8th day— one unfit for dissection. Two killed on the 8th day— one infected. |

The infected female was killed on 20th June, 1920; the gut contained four large oöcysts, the largest 60.7μ by 52.4μ , the smallest 51.0μ by 48.4μ , all with sporozoites.

In this experiment the females died early, and on examination, within an hour or two of death, their abdomens were crowded with yeasts and bacteria and the contents macerated. On the eighth day two females still survived, but to ensure reliable dissections they were killed. Infection of the gut was observed in one of them.

SUMMARY

I. Of twelve females of *Anopheles plumbeus* fed once on one or other of two cases of simple tertian malaria (*P. vivax*), and subsequently kept at room temperature (max. 22°C. , min. 13°C.) none became infected.

II. Of twelve females of *Anopheles plumbeus* fed once on a case of malignant tertian malaria (*P. falciparum*), and subsequently kept at 28°C. , none lived longer than eight days after the infected feed; one contained oöcysts in the mid-gut.

APPARATUS AND TECHNIQUE

The article referred to above (1920) in connection with the experimental work also contained a short account of the technique adopted at that time; but this has since been considerably improved upon, and in view of its importance in mosquito-infection work in the laboratory, we have here embodied a description of the methods and apparatus used during the period in which the experiments recorded were carried out.

Larvae in various stages were collected from rot-holes in trees in the vicinity of Liverpool, mainly during the months of March and April, 1920. They were brought to the laboratory in glass jars and placed in inverted bell-jars (mounted on wooden stands) containing water and sediment from the tree-holes. Low, wide-mouthed bell-jars (6 inches deep, 12 inches diameter) were found most convenient, and were filled to a depth of about two or three inches with the water and sediment. Numbers of larvae were maintained in each jar by judiciously supplying them with dry, powdered cockroaches; they fed voraciously on this diet, and the rate of their development could

apparently be controlled by the frequency of its application. Every morning the larval jars were examined, and all pupae* removed to small glass containers mounted on wooden trays. The trays consisted of wooden platforms ($\frac{3}{4}$ -inch thick) raised on runners, two and a half inches high, at each end, and pierced in eight places by circular holes to carry the pupal containers. The dimensions of the trays most often used were length 27 inches, width 15 inches, but smaller one (18 inches by 10 inches) were also available, though found to be of less convenient size. The pupal containers used in the larger trays were small potted-meat jars of a common pattern (internal diameter $2\frac{1}{2}$ inches, depth about $1\frac{1}{2}$ inches) provided with thick laterally projecting rims or flanges, and were sunk into the trays in such a way that the rims were flush with the platforms. Each of the larger trays was provided with a double row of four evenly-spaced containers, so that each of the latter was in the centre of an area of the platform approximately nine inches square, and separated from the edge of the tray or the next jar by about three inches. To avoid overcrowding, not more than ten or a dozen pupae were placed in one jar, but not infrequently less than this number was present since the daily supplies of pupae were kept separate. If much larger numbers of pupae were placed in the containers or if young and old pupae were indiscriminately mixed, the mortality, particularly among emerging adults, was found to be increased. Even when the greatest care was exercised, partially or completely emerged adults were sometimes found drowned, although the employment of small 'bridges' of thick paper (renewed daily) apparently rendered the process less perilous. Immediately after the transference of pupae from the larval jars to the trays, the containers were covered with glass cylinders, the upper ends of which were closed by pieces of mosquito-netting attached by rubber bands. Considerable difficulty was at first experienced in obtaining cylinders of suitable sizes and at reasonable cost, but subsequently this was overcome by using glass engine-gauges. These are made in a variety of sizes of very thick, but clear glass with ground edges, and are easily procurable and altogether satisfactory for the purpose

* The material collected from the rot-holes naturally included large numbers of larvae of *Ocblerotatus geniculatus*; the pupae of this species were separated from those of the *Anopheles* with ease during the daily segregation and thus a protracted search for minute larvae avoided.

required. The cylinders selected for covering the pupal containers were four inches in length with an internal diameter of three and a half inches. The adults were removed from these each morning, and the females and a few males transferred to narrower cylinders (5 inches long, $2\frac{1}{2}$ inches diameter) more suitable for feeding purposes. Transference was accomplished by slipping a piece of stiff paper under the large cylinder on the pupal tray and removing it bodily to a simple apparatus which enabled the insects to be blown from one cylinder to the other. This apparatus was formed of two platforms of stout cardboard, wired to retort-rings fitted on a stand; one of the platforms was placed above the other, and consisted of two superimposed sheets of cardboard (approx. 10 inches by $6\frac{1}{2}$ inches) fastened together at their sides but slightly separated from one another and pierced in the middle by a circular aperture 2 inches in diameter. A third and slightly narrower sheet of cardboard was pierced in a similar manner, but nearer to one end; it could slide between the other two pieces, and, if pushed home, close the apertures in the upper and lower sheets. The lower platform consisted of a single sheet of cardboard, and acted as a moveable tray for the lower and smaller cylinder into which the mosquitoes were to be transferred. The latter was first screened at one end, and then the open end adjusted to the aperture on the under side of the upper platform; the large cylinder containing the mosquitoes was placed on the upper side of the platform over the aperture and the paper slipped from beneath it. The sliding sheet was then pulled out a distance sufficient to allow its aperture to coincide with those of the upper and lower sheets forming the upper platform, and the insects blown downward. For this purpose it was found advisable, first to cause the mosquitoes to fly about and then to direct them towards the aperture by blowing through a piece of rubber tubing. When all the mosquitoes had entered the lower cylinder the sliding sheet was pushed home and the cylinder removed, after first slipping paper between its open end and the upper platform. Mosquito-netting was then fixed over the open end, and the cylinder subsequently placed on damp filter paper in a petri dish. Except the single meal of blood received from the patient, the adults were fed only on split and moistened raisins, to which they always had access; the raisins were placed upon the

netting covering the upper ends of the cylinders, and were renewed every three or four days. From fifteen to twenty mosquitoes, chiefly females and of nearly the same age as possible, were kept as stock insects in each of these feeding-cylinders, in a shaded corner of the room or in a cupboard. When a suitable case presented itself, one of the gauze-covered ends of the cylinders was applied to the skin, usually the inside of the fore-arm, and allowed to remain until most or all of the females had engorged. *A. plumbeus* bites readily, and it was not found necessary to shade the cylinders during this operation; but, in order to induce as great a number as possible of the females contained in the cylinder to engorge rapidly, that portion of the patient's skin where feeding was to take place was usually wiped with a hot, damp cloth, and the cylinder occasionally inverted so that females which had settled on the gauze covering the upper end came into actual contact with the skin and were thus stimulated to bite. The mosquitoes were then placed in the incubator or left in the room according to the conditions of the experiment. Subsequently they were removed from the cylinders when required for dissection. In most of our work, after the lapse of four or five days from the blood meal, one or two females were dissected daily. Their separation from the other females and removal from the cylinder was accomplished by means of the transferring apparatus described above. A sliding sheet with a smaller aperture (one inch diameter) was, however, substituted for the one previously mentioned. The rubber blow-tube was attached to a six-inch length of half-inch glass tubing, of which that end inserted into the rubber tubing was covered with a piece of gauze. The cylinder, with paper instead of the gauze at the lower end, was placed on the upper platform of the apparatus, the sliding sheet pulled out so that the apertures coincided, and the glass-tubing thrust through the paper. A single female was then drawn into the tube by suction; the glass withdrawn and the sliding sheet pushed home. The mosquito was then killed with chloroform and dissected in the usual manner.

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- BLACKLOCK, B, and CARTER, H. F. (1920). The Experimental Infection in England of *Anopheles plumbeus*, Stephens, and *Anopheles bifurcatus*, L., with *Plasmodium vivax*. *Ann. Trop. Med. and Parasit.* Vol. XIII, pp. 413-420.

EXPLANATION OF PLATE IX

Apparatus used in experimental-infection work with mosquitoes and malaria :—

- A—Inverted bell-jars containing stock larvae.
- B—Pupal trays, showing the small pupae-containers and the screened cylinders for reception of emerged adults.
- C—Feeding-cylinders, screened at both ends with raisins on top, and standing in petri dishes on damp filter-paper; for stock adults and experimental females.
- D—Transferring apparatus, showing retort-stand with the two platforms fitted on the rings, and cylinders in position for the transferring process; also the rubber blow-tube with glass extension for isolation of experimental females.

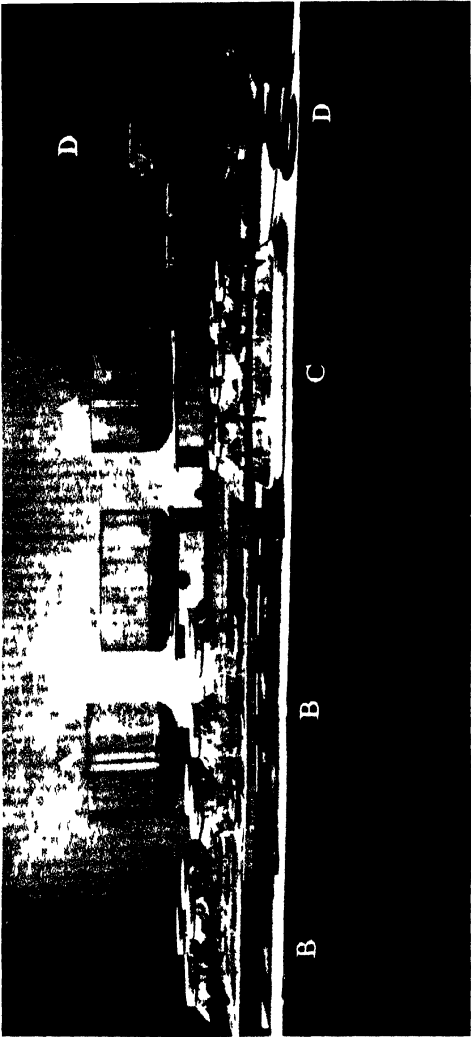


Photo by Miss M. Brown

HUMAN INTESTINAL PROTOZOA IN NORTH QUEENSLAND

BY

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From the Australian Institute of Tropical Medicine, Townsville

(Received for publication 1 October, 1920)

This investigation was undertaken with the object of ascertaining if carriers of *Entamoeba histolytica* exist among the healthy population of North Queensland, as in other parts of the world. This has been shown in England by Yorke and others (1917), and by Matthews and Smith (1919), and in Egypt by Wenyon and O'Connor (1917).

In the present series the number of people examined is five hundred, and the specimens were obtained from among a number of about one thousand five hundred, which were collected by members of the "Hookworm Campaign" staff, during their investigation of that disease. With the exception of seventy-one stools from the Townsville Orphanage, they were chosen quite at random. They include all ages of the community, from 1 year to 80 years of age, and embrace a wide area of country to the North and West of Townsville, within a radius of seventy miles of the town. All the houses are small, and built of wood or galvanized iron, and are not crowded together, in many cases miles separating them. The conservancy system is pans, and the privies are not fly-proof.

From their manner of collection, the specimens were at least three, and perhaps as much as fourteen, days old before being examined, therefore no vegetative forms of the various organisms were seen, but only their cysts. And for the same reason only one examination of each case was possible. This point should be borne in mind throughout the perusal of the paper, because it has been

shown by Carter and others (1917) that only about one quarter of the total *Entamoeba histolytica* infections are revealed at the first examination, and similarly one-third of the *Entamoeba coli*, and one-half of the *Lamblia intestinalis* infections only are found.

The following protozoal cysts were seen, viz.:—*Entamoeba histolytica* (Schaudinn), *Entamoeba coli* (Losch), *Lamblia* (*Giardia*) *intestinalis* (Lambl), *Tetramitus* (*Chilomastix*) *mesnili* (Wenyon). Other cysts of a vegetable nature were encountered, which are mentioned at the end of the paper.

General results. The total figures are given in Table I, and call for no special comment.

TABLE I.

| Type of infection | No. of stools examined | No. infected | Percentage |
|-------------------------------|------------------------|--------------|------------|
| <i>E. histolytica</i> | 500 | 23 | 4.6 |
| <i>E. coli</i> | 500 | 134 | 26.4 |
| <i>L. intestinalis</i> | 500 | 59 | 11.8 |
| <i>T. mesnili</i> | 500 | 11 | 2.2 |
| '? cyst' | 500 | 9 | 1.8 |

Mixed infections occurred as follows:—

E. histolytica, *E. coli*, and *L. intestinalis*, five times.

E. histolytica and *E. coli*, five times.

E. coli and *L. intestinalis*, sixteen times.

E. coli and ' ? cyst,' three times.

E. coli and *T. mesnili*, once.

For purposes of comparison, Table II has been compiled from a paper by Carter and others (1917), with the addition of the figures given in a series of examinations by Matthews and Smith (1919), and those of the present paper.

TABLE II.

| | | | Present series | Matthews and Smith (1919) | Carter and others (1917) | Wenyon (1915) | Dobell (1916) | Jacobs (1916) | Matthews and Smith (1917) |
|----------------------------|--------------|-----|----------------|---------------------------|--------------------------|---------------|---------------|---------------|---------------------------|
| | | | 1 | 2 | 3 | 4 | 5 | 6 | 7 |
| No. examined | Dys. ... | ... | ... | ... | 910 | 556 | 90 | 153 | ... |
| | Non-dys. ... | ... | 500 | 450 | ... | ... | 110 | 204 | 250 |
| Total examined ... | | | 500 | 450 | 910 | 556 | 200 | 426 | 250 |
| <i>E. histolytica</i> ... | | | 4.6 | 1.5 | 10.3 | 10.8 | 11.0 | 7.7 | 8.0 |
| <i>E. coli</i> ... | | | 26.8 | 6.7 | 25.4 | 39.0 | 40.9 | 25.8 | 19.2 |
| <i>L. intestinalis</i> ... | | | 11.8 | 6.0 | 18.6 | 16.0 | 19.5 | 19.0 | 8.0 |
| <i>T. intestinalis</i> ... | | | ... | ... | 1.2 | 1.6 | 2.4 | ... | 1.7 |
| <i>T. mesnisi</i> ... | | | 2.2 | 1.5 | 2.7 | 0.5 | 7.8 | ... | 2.0 |

All the figures in the above table are in percentages. It must be borne in mind that columns 1 and 2 give the results of a single examination, and the remaining columns are the aggregate of repeated examinations of each case.

The objection may be raised that some of the figures refer to dysenteric, and others to non-dysenteric cases. But Wenyon and O'Connor (1917) have shown that there is very little difference between the two classes of case, as far as percentage of carriers is concerned. This fact is well sustained in the table given above.

It is obvious, allowing for the single examination, that the figures in the present series are high, especially in regard to *E. histolytica*, *E. coli*, and *L. intestinalis*. But when compared with those of a series of examinations by Matthews and Smith (1919), in which they made one examination only, of a number of

non-dysenteric cases, it is seen that they are well within the bounds of the figures for England.

This is shown in Table III, in which all the figures are in percentages, and are the result of a single examination of healthy people in every case.

TABLE III.

| | No. examined | <i>E. histolytica</i> | <i>E. coli</i> | <i>L. intestinalis</i> | <i>T. mesnili</i> |
|-----------------------|-----------------|-----------------------|----------------|------------------------|-------------------|
| Present series | 500 | 4·6 | 26·8 | 11·8 | 2·2 |
| Batch 1 | 263 | 3·0 | 12·1 | 4·5 | ... |
| Batch 2 | 241 | 6·6 | 23·2 | 7·5 | 1 case |
| Batch 3 | 230 | 3·9 | 14·8 | 6·1 | ... |
| Batch 4 | 98 | 6·1 | 21·4 | 8·2 | ... |
| Batch 5 | 84 | 8·2 | 22·4 | 11·8 | 1 case |
| Batch 6 | 104 | 5·8 | 17·1 | 6·7 | ... |
| Batch 7 | 78 | 12·8 | 23·1 | 10·2 | ... |

Age incidence. The effect of age on the incidence of infection is shown in Table IV. It is in agreement with the findings of Matthews and Smith (1919), and supports the tentative view expressed by them in this connexion, that the proportion of young people infected is higher than that of adults. The present investigation covers a much wider range of ages than that of the above observers, as it embraces people from 1 year to 80 years. Nevertheless, it is open to the objection, that the numbers in the various age-groups are somewhat small, and, consequently, do not give quite accurate results, which would be obtained if a higher number were examined. This is especially noticeable in the Ophanage group 1 to 5 years, with only nine cases, and three infections by *E. coli*, thus giving a percentage of 33·3 per cent., which is obviously much too high. The fact of the relatively high incidence of *L. intestinalis* among children is well shown. This is also pointed out by the above workers.

In this, and all subsequent tables, *T. mesnili* and ' ? cyst ' are not shown, because the actual number of these infections is too small for such comparison.

TABLE IV.
(PEOPLE IN PRIVATE HOUSES)

| Age group in years | No. examined | Percentage infected | | |
|--------------------|--------------|-----------------------|----------------|------------------------|
| | | <i>E. histolytica</i> | <i>E. coli</i> | <i>L. intestinalis</i> |
| 1-5 | 48 | 4'2 | 4'2 | 22'9 |
| 6-10 | 55 | 5'5 | 12'7 | 23'6 |
| 11-15 | 41 | 9'75 | 41'5 | 22'0 |
| 16-20 | 39 | 5'1 | 23'1 | 10'2 |
| 21-30 | 72 | 6'9 | 29'2 | 4'2 |
| 31-40 | 57 | 1'75 | 26'3 | 8'8 |
| 41-80 | 117 | 1'7 | 23'9 | 5'1 |

(ORPHANAGE CHILDREN)

| | | | | |
|-------|-----|------|------|------|
| 1-5 | 9 | 0'0 | 33'3 | 11'1 |
| 6-10 | 42 | 4'8 | 50'0 | 14'3 |
| 11-15 | 200 | 10'0 | 55'0 | 20'0 |

In the above table it will be noted that the Orphanage has been given separately. This is because 33 per cent. of all the children examined live in it, and as they are living with only one or two adults, it is not quite the same as the children living in private houses with a relatively large number of adults.

Sex incidence. The equality with which the three types of organism under consideration is distributed between the sexes is remarkable, and is shown in Table V.

TABLE V.

| Sex | No. | <i>E. histolytica</i> | <i>E. coli</i> | <i>L. intestinalis</i> |
|---------------|-----|-----------------------|----------------|------------------------|
| Male | 274 | 4'9 | 25'1 | 12'8 |
| Female | 218 | 4'5 | 23'4 | 11'9 |
| Sex? | 8 | ... | ... | ... |

Matthews and Smith (1919), in a small series of seven houses, showed that when one person in a given house is infected, the other members of the same household show a relatively high percentage of infection. The same has been worked out for the present series, and is shown in Table VI.

On account of the large number of houses from which cases have been examined, the details of infection are not given for each house separately. They are shown as average percentages of the infected population. This figure was obtained by working out the percentage of people infected in each house in which infection occurred, and then striking the average of these percentages.

The Orphanage is again given separately, because with its population of seventy-one it would have an undue influence on the totals of the private houses, whose average population per house is only three to four.

TABLE VI.

PRIVATE HOUSES

| Type of infection | Total No. of houses examined | No. of houses infected | Percentage | Total population of infected houses | No. of population infected | Average percentage infected per house |
|-------------------------------|------------------------------|------------------------|------------|-------------------------------------|----------------------------|---------------------------------------|
| <i>E. histolytica</i> | 158 | 13 | 8'2 | 64 | 19 | 39'5 |
| <i>E. coli</i> | 158 | 66 | 41'8 | 223 | 99 | 54'2 |
| <i>L. intestinalis</i> | 158 | 25 | 15'8 | 95 | 48 | 54'3 |

ORPHANAGE

| | | | | | | |
|-------------------------------|---|---|-----|----|----|------|
| <i>E. histolytica</i> | 1 | 1 | 100 | 71 | 4 | 5'6 |
| <i>E. coli</i> | 1 | 1 | 100 | 71 | 35 | 49'3 |
| <i>L. intestinalis</i> | 1 | 1 | 100 | 71 | 11 | 15'5 |

It appears in the above table that, including the Orphanage, there are one hundred and five houses with infections of one kind or another in them. But the actual number of houses containing infections is only seventy-five, because some houses have two or all types of infection in them. Similarly among the people two hundred

and sixteen infections are distributed between one hundred and eighty-five individuals.

Further, it is noticed that the figures in the last column are higher than the percentages which would result from comparing the total population of infected houses and the number of population infected. This is because some houses with only one inhabitant, who is infected, tend to raise the average.

There is no apparent reason why the Orphanage figures do not more closely agree with the averages for the private houses. Nevertheless, they are distinctly higher than the total infections found, as will be seen by comparing them in Table VI with those of Table I.

The fact of the equal liability of the sexes to infection and that of the high incidence of infection in houses which contain infected people, when taken together seem to be of some value, as an indication of the probable mode of spread of infection.

All the adult males are employed in outdoor work, and the females are occupied in house work. Therefore, the infection most likely occurs in the neighbourhood of houses, as outdoor work has no apparent influence on the infection rate, and vice-versa.

Wenyon and O'Connor (1917) in a series of experiments proved that house-flies can act as the carriers of cysts of *E. histolytica*, and when it is remembered that in the houses under consideration all the privies are of the dry-earth type, and by no means fly-proof, the idea of flies being active agents in the spread of infection, at all events in this district, is worthy of consideration.

Origin of the infection. As a result of inquiries among the twenty-three carriers of *E. histolytica*, it is found that, with one exception, they have all lived in their present houses many years, and in most cases all their lives. The one exception was at the war for three years, but never had dysentery or diarrhoea. All except one, who was born in Ireland, and has been in Australia twenty-seven years, were born in this country, and have not been out of it, except the soldier.

Two of them have had attacks of dysentery, both some years ago, one in the present abode, and one in Western Australia. The true nature of these attacks is not obtainable.

No work has been done on returned Australian soldiers to find

if there are carriers of *E. histolytica* among them. Nevertheless, it is practically certain they exist, because carriers in the British army have been found by Wenyon (1916), Jepps (1916), Matthews and Smith (1917), Turner and Taylor (1919), and many others. And the same has been found in the American army by Kofoid and others (1919). Australian soldiers were living under identical conditions as the soldiers of the above two armies, and, further, many of them had dysentery. It is, therefore, reasonable to assume that there are carriers among them. But H. Johnston (1909) has collected records of six or seven cases of amoebic dysentery or liver abscess occurring in Australia prior to that date. Therefore, *E. histolytica* has probably existed in Australia for many years, and, as in many other countries, only occasionally causes disease. Consequently, the almost certain introduction of a large number of carriers among the returned soldiers need not necessarily be followed by an increase in incidence of the disease.

Special considerations. *E. histolytica* has shown nothing worthy of special notice. *E. coli*: Small cysts of this organism have been relatively common. By small cysts is meant those from 11μ to 14μ . This in a total of one hundred and thirty-four infections, a high proportion, as shown by Smith (1919). *L. intestinalis* has been found about an equal number of times in each of the two types described by Matthews (1918), viz., the usually described, brown-staining variety in iodine, and the smaller bluish type described by the above observer. They either occurred alone or both in the same stool. *T. mesnili* in some stained preparations showed the chromatin of the nucleus evenly distributed around the nuclear membrane, and hence did not present the 'signet-ring' type of nucleus described by Matthews (1918) as typical. *Entamoeba nana* (Wenyon) was not found at all. This is somewhat strange, as it seems to occur in most other parts of the world, in company with the protozoa found at this examination. But the number of cases examined is too small on which to make a definite pronouncement as to its presence or absence in Australia, and it will very likely appear at subsequent examinations. *I. cysts* (Wenyon) were encountered five times. In one case these cysts, with the iodine vacuole distinct, were seen at the serial examination. But when the same case was examined after treatment for *Agchlystoma duodenale*,

large numbers of the same cysts were seen, but on this occasion none showed the iodine vacuole.

Blastocystis hominis was fairly common, more especially when reasonably fresh stools were examined. The actual number of times it occurred was not recorded. A single rather interesting observation in connection with this cyst has been noted. A case that showed large numbers of *I. cysts* in a formed stool at the serial examination was again examined after treatment for *A. duodenale*, when the stool was liquid. On the second examination no *I. cysts* were found, but the stool was full of *B. hominis*, none of which were found at the first examination. Wenyon (1917) mentions the development of *B. hominis* and diarrhoea concurrently in a case he was controlling for *E. coli*, and suggests a possible connection between the two, but draws no definite conclusion. The above case, observed at Townsville, raises the question whether *B. hominis* may not appear because of the diarrhoea artificially induced. And the same thing may have occurred in Wenyon's case, although there the diarrhoea was not brought on by the use of drugs. But O'Connor (1919), in a later paper, seems to trace some connexion between *B. hominis*, *I. cysts*, and some other vegetable cysts, and a special form of diarrhoea of which he has observed fifteen cases.

'? cysts.' In fresh preparations, both in saline and iodine solution, this cyst was to all appearance very like *E. nana*. It is a small cyst varying in shape from a distinct oval to one almost circular. It is 5μ to 12μ in its long diameter, and contains a few refractile granules, which might easily be mistaken for indistinct nuclei, such as are seen in *E. nana*. But in preparations stained with iron-haematoxlyn the granules are seen as small, darkly-staining dots, which are quite homogeneous and show no nuclear structure. They vary from one to seven in number. After prolonged search of stained preparations a few budding forms were found, thus definitely proving its vegetable origin. Attention has only been drawn to it because of its likeness to *E. nana*.

Eggs of the following intestinal worms were found during the course of the examination, viz. :—*Agchylostoma duodenale*, *Oxyuris vermicularis*, *Trichuris trichiura*, and those of a *Hymenolepis* sp. (probably *H. nana*). The larvae of *Strongyloides stercoralis* were fairly often encountered.

The number of times these infections occurred is not given because many are missed when the stools are not centrifugalised, and, further, they will be published in full, and for a much greater number of cases, in the reports of the 'Hookworm Campaign.'

SUMMARY

The protozoa of the human intestine are substantially the same in Australia as in other parts of the world where similar examinations have been carried out, and they occur in approximately the same proportion of cases, among healthy people.

Confirmation is given to the view that the incidence is higher in children and young adults than in people of more mature age.

Evidence has been put forward to show that flies are very probably carriers of the infection.

In conclusion, I wish to express my thanks to Dr. Breinl for his readiness always to discuss doubtful cases, and also for his assistance and suggestions in connection with the literature; and to Dr. Willis, of the 'Hookworm Campaign,' and the members of his staff, for free access to their material, and for obtaining for me any information I required from any of the cases examined.

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A NOTE ON THE OCCURRENCE OF CERTAIN CESTODES IN NEW HOSTS

BY

T. SOUTHWELL

(Received for publication 15 October, 1920)

In going through the collection of Cestoda in the Liverpool School of Tropical Medicine, the following were found in new or unusual hosts:—

1. *Avitellina centripunctata*, Rivolta, 1874.
 = *Taenia centripunctata*, Rivolta, 1874.
 = *Stilesia centripunctata* (Rivolta), Stiles, 1893.
 = *Stilesia centripunctata* (Rivolta), Railliet, 1893.
 = *Stilesia centripunctata* (Rivolta), Gough, 1909.

This worm occurs normally in sheep. Ward obtained it from *Bos taurus* in 1895. Numerous specimens of this worm were collected from the stomach of an ox by Professor Yorke, 1915, in the military slaughter-house, Freetown.

2. *Stilesia hepatica*, Wolffhügel, 1903.
 = *Stilesia hepatica* (Wolffhügel), Gough, 1908.
 = *Stilesia sjostedti*, Führmann, 1909.

This species has previously been obtained from sheep, goats, and wild ruminants.

Our collection contains specimens from the following hosts:—

(a) Ox stomach. Several specimens collected by Professor Yorke from the European slaughter-house, Freetown, December, 1914.

(b) From cattle. Several specimens collected by Dr. J. W. S. Macfie, Accra, Gold Coast, September, 1915.

(c) From buffalo's stomach. Several specimens collected by Professor Newstead and Dr. Davey, Upper Shiré, Nyasaland, 1911.

(d) From a duiker (*Cephalophus monticola*). Two specimens collected by Professor Yorke, N.E. Rhodesia, July, 1912.

3. *Stilesia globipunctata* (Riv., 1874), Railliet, 1893.

This species is found in sheep and goats. We have several specimens from the bile ducts of a water-buck, *Cobus ellipsiprymnus*; collected by Dr. A. Kinghorn in Rhodesia, 1908.

4. *Moniezia oblongiceps*, Stiles and Hassall, 1893.

This species usually occurs in sheep, and has been found in *Coassus* sp. We have two specimens and some fragments from a water-buck, *Cobus ellipsiprymnus*, collected by Professor Yorke at Ngoa, N.E. Rhodesia, in July, 1912.

5. *Moniezia expansa* (Rud., 1810), R. Blanchard, 1911.

This species occurs in sheep, cattle, deer and other ruminants. We have fragments from a duiker, *Cephalophus monticola*, collected by Professor Yorke in N.E. Rhodesia, in July, 1913.

6. *Metroliathes lucida*, Ransom, 1900.

This species has been recorded from turkeys and chickens. We have several specimens from guinea-fowls (*Numida ptilorhyncha*); collected by Professor Newstead and Dr. J. B. Davey, Upper Shiré River, Nyasaland, 1911.

7. *Davainea tetragona* (Molin, 1858), R. Blanchard, 1891.

This species is found in chickens. We have a few specimens from guinea-fowls (*Numida ptilorhyncha*), collected by Professor Newstead and Dr. J. B. Davey, Upper Shiré River, Nyasaland, 1911, and also by Dr. Arnold in the Transvaal.

8. *Davainea cesticillus* (Molin, 1858), Blanchard, 1891.

This species has hitherto been recorded only from chickens and turkeys. We have two specimens from guinea-fowls (*Numida ptilorhyncha*); collected by Professor Newstead and Dr. J. B. Davey, Upper Shiré River, Nyasaland, 1911.

9. *Taenia* sp. (*saginata* ?).

Numerous fragments from a goat, *Capra cylindricornis*; collected by Dr. J. W. S. Macfie, Accra, Gold Coast, 1919.

No head was present. The fragments consisted of one hundred and fifty-five segments, each measuring, on an average, 7.5 mm. long and 4.5 to 5.5 mm. broad. Only about thirty of these segments contained genitalia, the rest being sterile, and longer and narrower than the segments containing genitalia. The genital pores were irregularly alternate and situated laterally, slightly posterior to the middle. The genital organs resembled, exactly, those of *T. saginata*. The main stem of the uterus was long and somewhat narrow, and had from fourteen to nineteen lateral branches.

Eggs. No mature eggs were found, and measurements cannot therefore be given.

The occurrence of a species of *Taenia* in a goat calls for comment, for, as far as we are aware, no species of this genus has hitherto been recorded from a ruminant. Our fragments were obtained by Dr. Macfie from a goat, killed in the slaughter-house at Accra. There can be no doubt that the occurrence of this parasite in a goat is accidental; this opinion is corroborated by the presence of numerous sterile segments, for it is well known that the parasites found in unusual hosts tend to become sterile.

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ERRATUM

Vol XIV, p 296, line 16 For '*Metroliathes lucida*' read
' *Metroliaesthes lucida* '

TWO NEW *CYLICOSTOMUM* SPECIES FROM THE HORSE

BY

DR. ALEXANDER KOTLÁN

*From the Royal Hungarian Veterinary College, Budapest**(Received for publication 19 October, 1920)*

INTRODUCTION

The Cylicostomes—worms of the Equidae (horse, donkey, mule and zebra)—comprise very numerous species. Our knowledge of the various forms has increased, particularly during the last few years. At the present time, the known species of the genus *Cylicostomum* are twice as numerous as those described by Looss in 1901. This fact, which is of great systematic interest, must be a starting point for anatomical, histological and biological studies concerning the life-history, and especially the position of the various forms within the genus *Cylicostomum*. Looss (1901) discusses in his important monograph on the 'Sclerostomes of Horses and Donkeys in Egypt' the possibility of the division of the genus *Cylicostomum*; he found that with the exception of some isolated forms, the majority of the species could be classified in certain groups. Similarly, the new species which have been described since the appearance of Looss' work can also be classified; the majority can be referred to one or other of Looss' groups, but the number of isolated forms is increased.

Recently, new genera have been erected for certain forms belonging to the genus *Cylicostomum*, s.l., namely, *Poteriostomum* (Quiel) = *Hexodontostomum* (Ihle) and *Craterostomum* (Boulenger); but *Poteriostomum* must be considered only as a variety of *C. ratsii*, and since the chief characters of both forms do not differ essentially from those of the genus *Cylicostomum*, I think that the establishment of this new genus (*Poteriostomum*) is not justified. Moreover, *C. ihlei*, sp. n., which has undoubtedly the general

characters of the genus *Cylicostomum*, should also be referred on account of the structure of its mouth capsule to the genus *Poteriostomum*; this, however, seems to me incorrect.

Concerning the new genus *Craterostomum* (Boulenger), I think that this is very closely related to *C. acuticaudatum*, mihi = *C. mucronatum* (Ihle), and is perhaps even identical with the latter; but it must be observed that the characters of *C. acuticaudatum* correspond to those of the genus *Cylicostomum*.

In the following pages I give a brief description of two new species of the genus *Cylicostomum* found in the large intestines of Hungarian horses.

Cylicostomum ihlei, sp. n.

This new species, named in honour of Prof. I. E. W. Ihle (Utrecht), exhibits, as regards the structure of the mouth capsule, a decided resemblance to *C. ratzi*, mihi. The size of the worm is generally smaller than in the mentioned species. The males were

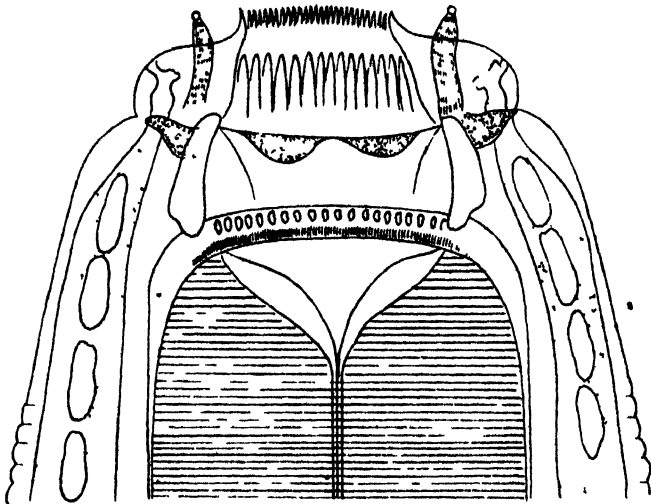


FIG. 1. *Cylicostomum ihlei*, sp.n. Anterior extremty, dorsal view. $\times 180$

from 9 mm. to 10 mm. long, the females at most 14 mm. long; with a maximum thickness of 595μ in the male and 900μ in the female. The constriction separating the mouth collar from the body is feebly marked; mouth collar about as high (40μ to 43μ) as it is thick.

The submedian head papillae are conical, their extremities rounded and notched, their length nearly 18μ ; lateral papillae large and not projecting beyond the surface of the mouth collar. The mouth opening is circular.

The external leaf-crown, originating from the posterior margin of the mouth collar, consists of about sixty slender and sharply-pointed

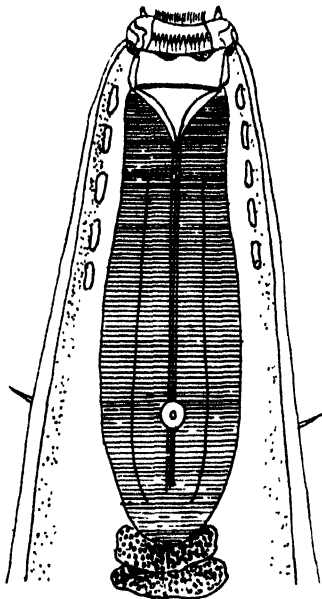


FIG. 2. *Cylicostomum iblei*, sp.n. Anterior end of the body, ventral view. $\times 90$.

leaves, similar in shape to those of *C. ratsii*, mihi. The internal leaf-crown, composed of about forty to forty-six large pointed leaves, arises from immediately behind the anterior border of the mouth capsule; the elements of the internal leaf-crown have in general a similar shape and appearance to *C. ratsii*, and their tips extend forwards beyond the mid-plane of the mouth collar.

The mouth capsule is nearly cylindrical, its posterior diameter is hardly greater than the anterior; the maximum breadth is 148μ , and the maximum depth 67μ ; the walls slightly increase in thickness from before backwards. In certain worms belonging to this species,

the external surface of the anterior margin of the mouth capsule is characterised by the presence of eight semi-circular (in dorsal or ventral view) ledges,* their arrangement is regular, namely: between every two submedian head-papillae there is a pair of ledges, and between every lateral and submedian head-papillae a single ledge. A similar peculiarity exists also in *C. acuticaudatum* (Kotlán, 1919) = *C. mucronatum* (Ihle, 1920). Dorsal gutter absent.

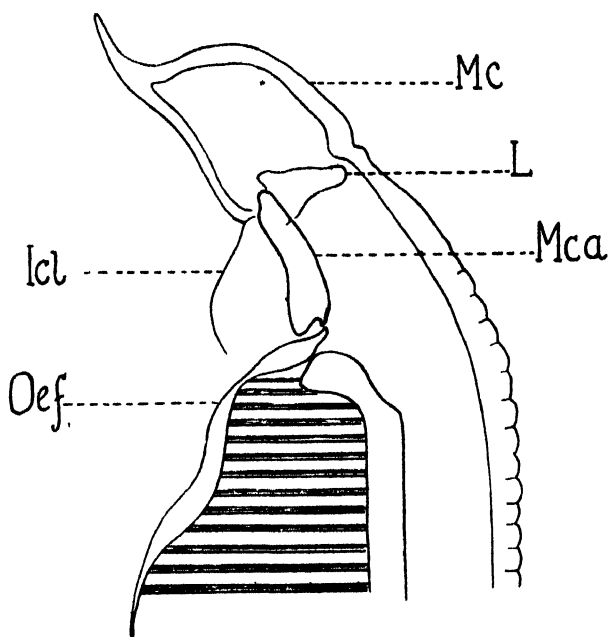


FIG. 3. *Cylicostomum siles*, sp.n. Optical longitudinal section of mouth collar, mouth capsule and adjoining part of the oesophagus. $\times 345$. Icl = internal cuticular lining; L = semi-circular ledge; Mc = mouth collar; Mca = mouth capsule; Oef = oesophageal funnel.

The oesophagus has a length of 680μ to 765μ ; it is almost cylindrical in shape up to the nerve ring, and behind this structure it slightly increases in thickness up to a maximum of about 200μ to 253μ . Cervical papillae and excretory pore lie over the posterior third of the oesophagus.

* These thickenings are entirely absent in certain specimens of these worms.

The posterior extremity of female resembles that of *C. elongatum* (Looss), but the base of the tail is stouter; the distance between vulva and anus is about 135μ to 148μ .

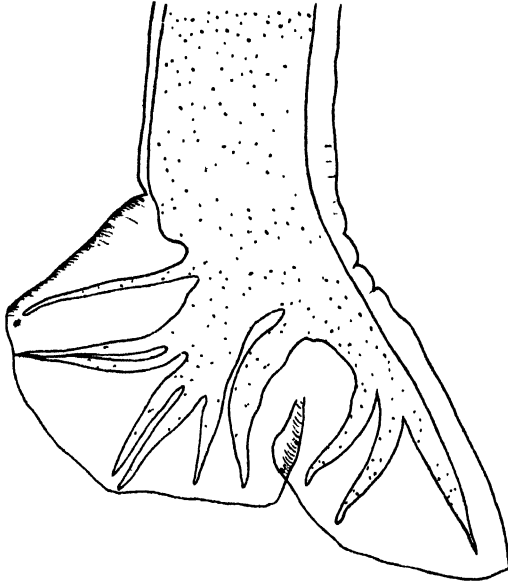


FIG. 4. *Cylicostomum iblei*, sp.n. Posterior extremity of male, lateral view. $\times 90$.

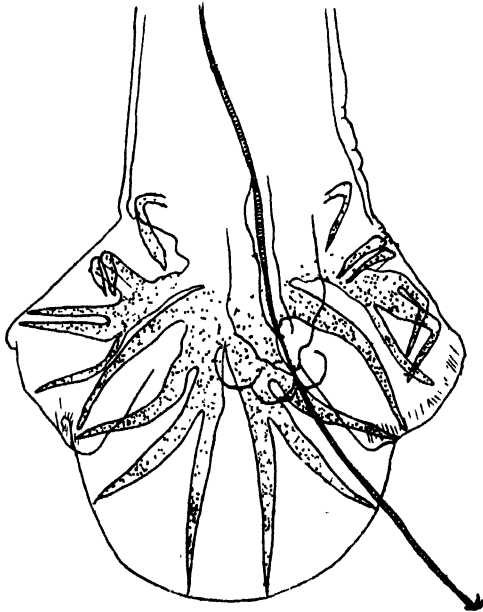


FIG. 5. *Cylicostomum iblei*, sp.n. Posterior extremity of male, ventral view. $\times 90$.

The bursa of the male has a moderate-sized median lobe; the lateral lobes are distinctly separated. The dorsal rays D_2 and D_3 are equal in length to D_1 ; the prebursal papillae are rather long.

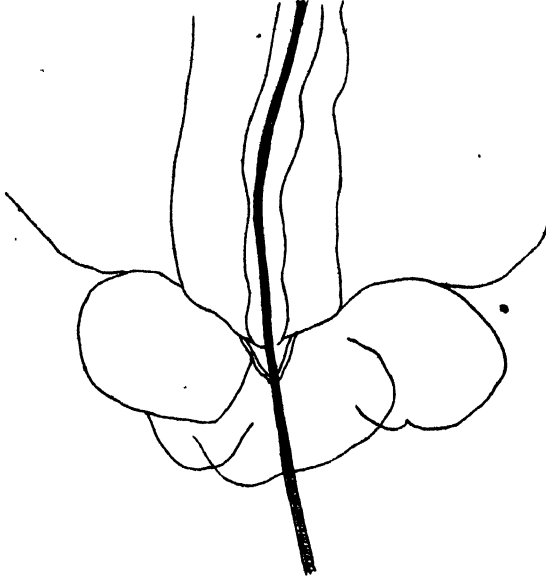


FIG. 6. *Cylicostomum iblei*, sp.n. Genital appendages, ventral view. $\times 180$.

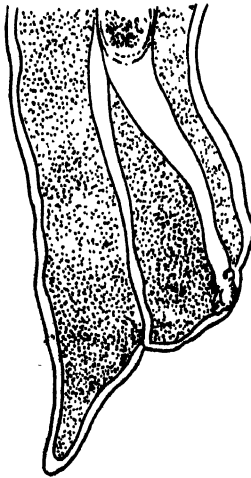


FIG. 7. *Cylicostomum iblei*, sp.n. Posterior extremity of female, lateral view. $\times 90$.

The genital cone and appendages are, in some respects, similar to those of *C. ratsii*:

C. ihlei is, owing to its peculiarities in the structure of the mouth capsule, particularly of the external and internal leaf-crown, very closely related to *C. ratsii*, but may be distinguished from this species by the size and shape of the submedian head-papillae, by the absence of a dorsal gutter, and in general by the entirely different shape and structure of the posterior extremity of the female. The close relationship of *C. ihlei* with *C. ratsii* is seen also in the rather similar structure of the genital cone and appendages in the two forms.

In the character of the mouth capsule *C. ihlei* closely resembles *C. euproctus* (Boulenger, 1917), but it can be distinguished from this species by the differences in the termination of the body in both sexes.

Cylicostomum prionodes, sp. n.

Three specimens (females) of this new form, belonging to the medium-sized cyclicostomes, were found in the caecum. The worms

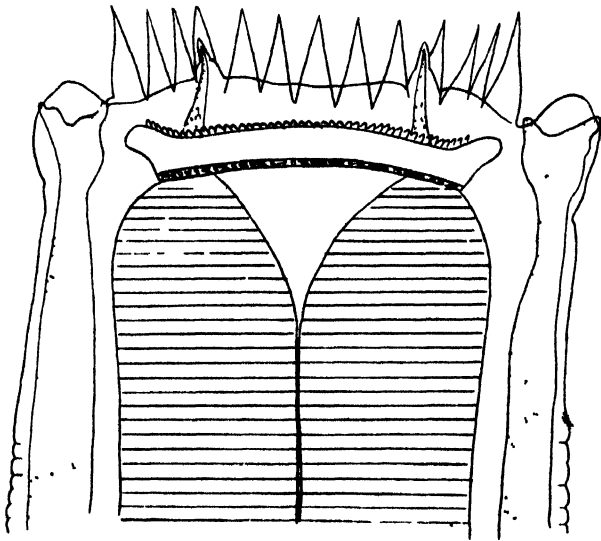


FIG. 8. *Cylicostomum prionodes*, sp. n. Anterior extremity, dorsal view. $\times 345$.

were from 10 mm. to 11.5 mm., with a maximum body breadth of 520 μ .

The bursa of the male has a moderate-sized median lobe; the lateral lobes are distinctly separated. The dorsal rays D_2 and D_3 are equal in length to D_1 ; the prebursal papillae are rather long.

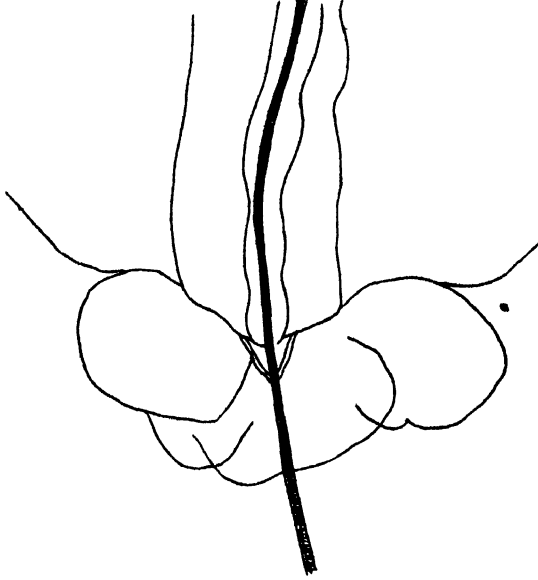


FIG 6. *Cylicostomum iblei*, sp. n. Genital appendages, ventral view $\times 180$.

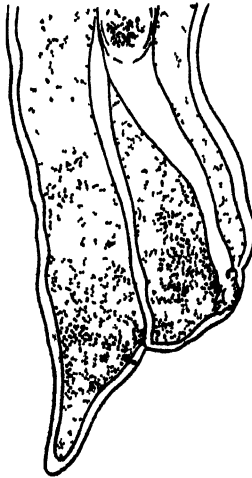


FIG 7. *Cylicostomum iblei*, sp. n. Posterior extremity of female, lateral view $\times 90$.

The genital cone and appendages are, in some respects, similar to those of *C. ratzii*:

C. ihlei is, owing to its peculiarities in the structure of the mouth capsule, particularly of the external and internal leaf-crown, very closely related to *C. ratsii*, but may be distinguished from this species by the size and shape of the submedian head-papillae, by the absence of a dorsal gutter, and in general by the entirely different shape and structure of the posterior extremity of the female. The close relationship of *C. ihlei* with *C. ratsii* is seen also in the rather similar structure of the genital cone and appendages in the two forms.

* In the character of the mouth capsule *C. ihlei* closely resembles *C. euproctus* (Boulenger, 1917), but it can be distinguished from this species by the differences in the termination of the body in both sexes.

Cylicostomum prionodes, sp. n.

Three specimens (females) of this new form, belonging to the medium-sized cylicostomes, were found in the caecum. The worms

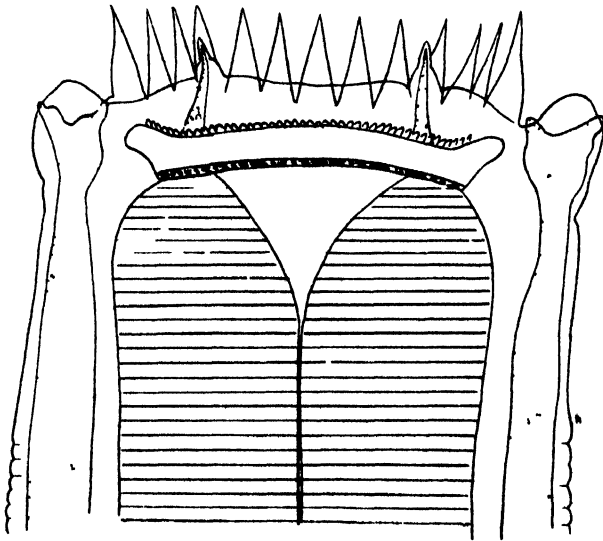


FIG 8 *Cylicostomum prionodes*, sp. n. Anterior extremity, dorsal view $\times 345$

were from 10 mm. to 11.5 mm., with a maximum body breadth of 520 μ .

Head continuous with the body. The mouth collar is distinctly marked from the rest of the skin, especially when seen laterally. The submedian head-papillae are pointed, rather long and prominent; the lateral papillae are rounded and not projecting. Mouth opening oval and very spacious.

The elements of the external leaf-crown resemble those of the teeth of a saw, numbering about twenty-four to twenty-six, arising from the posterior margin of the mouth collar. The length of these

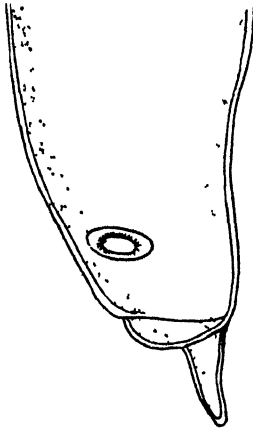


FIG 9. *Cylicastomum prionodes*, sp n. Posterior extremity of female, lateral view. $\times 90$.

elements is 32μ , their breadth at the base is 16μ . The internal leaf-crown consists of numerous small and inconspicuous elements originating from the anterior margin of the mouth capsule.

The mouth capsule is particularly shallow, having a depth of 13μ to 14μ . The dorso-ventral axis is shorter than the transverse, which measures about 90μ to 100μ . The walls of the mouth capsule are relatively thin.

The oesophagus is flask-shaped, 800μ to 935μ in length, with a maximum breadth in the posterior third of 172μ . Dorsal oesophageal gutter absent. The cervical papillae and excretory pore lie over the posterior third of the oesophagus.

The posterior extremity of female diminishes gradually up to anus; the tail is distinctly marked off from the body, and measures 125μ in length. The vulva is situated about 108μ from the anus.

Owing to lack of sufficient material, it is difficult to group

C. prionodes with any other species of the genus *Cylicostomum*. I believe, however, that this species is, in certain respects, allied to *C. brevicapsulatum* (Ihle, 1920), but is distinguishable from the latter by the shape of the mouth opening and of the mouth collar, and especially by the structure of the external and internal leaf-crowns.

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OBSERVATIONS ON THE CERATOPOGONINE MIDGES OF THE GOLD COAST WITH DESCRIPTIONS OF NEW SPECIES

PART III.

BY

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(*Received for publication 1 December, 1920*)

SYSTEMATIC ACCOUNT—*continued*

Genus *PRIONOGNATHUS*,* nov.

Eyes bare. Proboscis not longer than the head, chitinated, the mandibles (♀) serrated on both sides distally. Palpi composed of five segments, the second to fifth sub-equal in length, the fifth somewhat pyriform with an apical whorl of stout hairs; third segment not inflated, the sensory hairs long, arising from a very shallow, distal depression. Antennae of the female pilose, the five terminal segments somewhat elongate, of the male plumose, the three terminal segments elongate. Thorax arched anteriorly, not projecting over the head; anterior thoracic pits minute, posterior and post-scutellar pits absent. Wings entirely devoid of erect microscopic setae, but with sparsely arranged short, spine-like, decumbent hairs on the distal portion; anterior cross-vein short, first and third veins separate distally enclosing a single cell or interspace. Femora unarmed in the female, sometimes spinose in the male; first tarsal segment of middle leg distinctly longer and narrower than those of the fore and hind legs, of hind legs bearing, ventro-laterally, a

* *πρωον*, jaw and *γναθος*, jaw

double comb of short, strong bristles; fourth tarsal segment of all legs obchordate. Claws of female (at least one) very large, simple, equal or unequal; claws of male small, equal, bifid at the tips. Empodium minute, bristle-like.

Genotype *P. marmoratus*, sp. n.

This genus is allied to *Culicoides*, Latr., which it resembles somewhat closely in general morphology; but it is apparently more nearly related to *Alluaudomyia*, Kieff. (1913), and, indeed, is only separated therefrom by reason of the nature of the wings. In *Prionognathus* the wing surface is completely devoid of microtrichia (microscopic pubescence), while in the only known—and therefore type—species of *Alluaudomyia* (*A. imparunguis*, Kieff.), Kieffer states that the wings are covered with 'microscopic hairs and longitudinal ranges of longer hairs, some scattered longer hairs distally.' Long hairs (somewhat spine-like) are present on the distal portions of the wings of *Prionognathus*, but, although rather different in form from those observed in other genera, special importance cannot be attached to them since the distribution and density of arrangement of such hairs may show considerable specific variation—for example, in *Culicoides* the longer wing hairs may be numerous, sparse, or very scanty and confined to the distal margin. The presence or absence of microtrichia, however, appears to be a character of distinctly greater value; when such are present they are, so far as we have observed, distributed evenly over the wing field and specifically invariable.

Prionognathus is probably not confined to the tropics since the species described from Germany by Winnertz (1852) as *Ceratopogon splendidus* appears to be congeneric with the African specimens. Kieffer (1919) places this midge in *Culicoides*, but, so far as we are aware, it has not been captured since Winnertz's time, and the characters and figure of the wing (as well as the striking general facies of the species) given by the latter author seem to justify its inclusion in *Prionognathus*.

At present little information regarding the habits of these midges or of their early stages is available. It is probable, however, that the larvae, like those of *Dasyhelea*, are not strictly aquatic since a female of one of the species (*P. pseudomaculipennis*, sp. n.) obtained was reared from rotten wood taken from canoes.

The external morphology of this genus agrees, except as indicated in the generic description given above, with that of *Culicoides*, and, therefore, no detailed account is necessary. In two respects—the mouth-parts and wings—however, the description referred to may be extended.

Mouth-parts (fig. 1): Labium in both sexes, normal, soft and hairy. Labrum in the female, rather strongly chitinised, the proximal two-thirds broad, the distal third tapering to a somewhat pointed apex, fringed on both sides with delicate

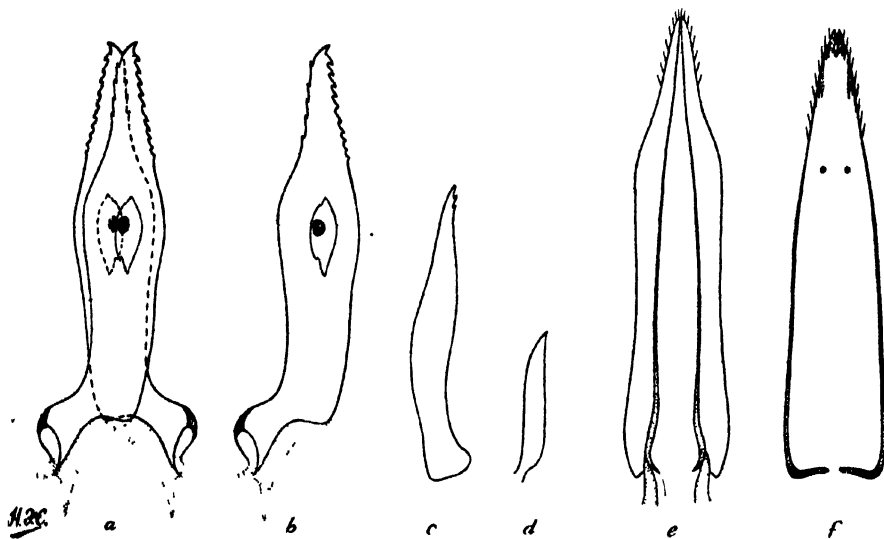


FIG. 1. *Prionognathus marmoratus*, sp.n. a—mandibles (♀), normal position; b—mandible (♀); c—mandible (♂); d—maxilla (♀); e—hypopharynx (♀); f—labrum (♀). ($\times 540$ circa.)

hair-like processes and armed with two large, terminal, and two pairs of smaller, lateral teeth; in the male, narrower, the distal half attenuated and devoid of teeth, but with longer and more numerous hair-like processes towards the apex. Hypopharynx less chitinised than the labrum, broad, tapering abruptly to a pointed apex or with the distal fourth attenuated and tapering to a narrowly rounded apex; distal hair-like processes apparently absent in some species. Mandibles* in the female, very strongly chitinised (except

* Judging by the arrangement of the mouth-parts in females mounted without pressure, the mandibles normally lie one over the other in the middle line, so that the inner edge of the one projects beyond the outer edge of the other (c.f. fig. 1, a). At first sight, therefore, a single, densely chitinised, median structure, armed with powerful recurved lateral teeth on the distal third, appears to be present.

near the centre where there is an oval, thinly chitinated area), broad, the distal third narrower, slightly tapering and armed on both sides with teeth—eleven on the inner edge, strong and, except the terminal one, directed backwards, five on the outer edge, smaller and directed forwards; in the male, smaller, narrower and less chitinated with three small teeth on the inner edge at the tip. Maxillae in both sexes rudimentary, reduced to very short, thinly chitinated, unarmed, blade-like processes.

Wings. In all the species described below the wings are hyaline, with the anterior veins cream-coloured, and with at least two small, irregularly outlined, blackish spots. The nature of these spots is peculiar; the wing membrane is granular and, under a high power of the microscope, the spots are seen to be formed by groups of pigmented granules with minute clear interspaces. The costal vein scarcely reaches the middle of the anterior border in the males, but extends slightly beyond it in the females, so that the position of the point of bifurcation of the fifth vein in its relation to the extremity of the costa, varies with the sex. The end of the third vein, just before its junction with the costa, is greatly thickened. The petiolate portion of the fourth vein is relatively long and clearly defined.

Prionognathus marmoratus, sp. n.

| MEASUREMENTS. | | | | | | Male. | Female. |
|--------------------------|-----|-----|-----|-----|-----|---------|---------|
| Length of body* | ... | ... | ... | ... | ... | 1.0 mm. | 1.3 mm. |
| Length of wing | ... | ... | ... | ... | ... | 0.8 mm. | 1.1 mm. |
| Greatest breadth of wing | ... | ... | ... | ... | ... | 0.3 mm. | 0.4 mm. |

Head dark brown, with brown hairs. Eyes in the female narrowly separate above; in the male rather more widely separate. Clypeus and proboscis dark brown, with scanty brown hairs. Palpi (fig. 2) dark brown, the second to fifth segments sub-equal, the fifth somewhat pyriform, with a distal whorl of about six moderately long, stout, hairs, third segment slender, not dilated, with a shallow, apical, depression from which arises a group of relatively long, knobbed, sensory hairs. Antennae brown, the first

* In all cases taken from anterior margin of thorax to tip of abdomen of specimens mounted in carbolic.

segment darker than the others; segments four to ten in the female, oval to sub-cylindrical, from about two to three times as long as the greatest width; last three segments in the male, elongate, sub-equal in length, the thirteenth slightly longer than the fifteenth; plume hairs mainly yellowish. *Thorax* pale coloured, marbled with darker markings. Dorsum grey or brownish-grey, becoming somewhat yellowish-brown laterally, with ring-like and crescentic sepia markings, most conspicuous on the antero-lateral portion; sparsely clothed with short brown hairs, and with longer, darker hairs

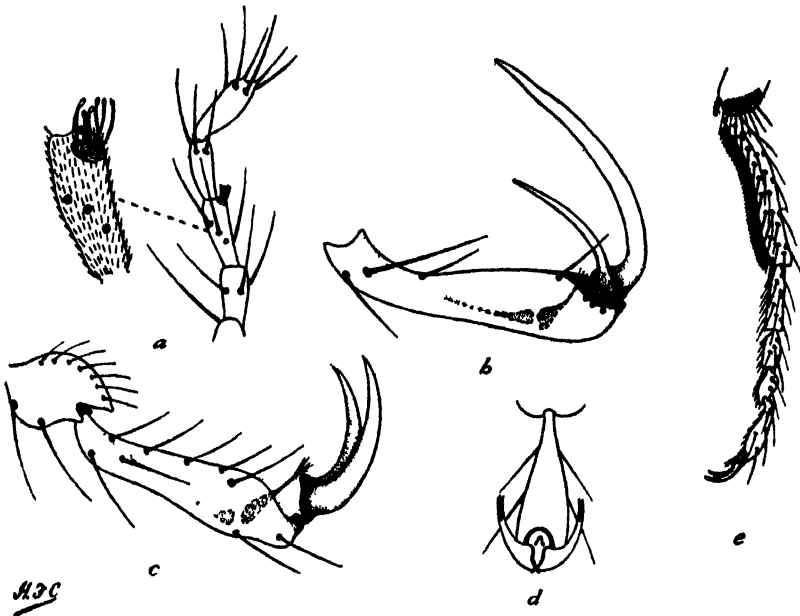


FIG. 2. *a*—*P. marmoratus*, sp.n., palp (♀); *b*—*P. maculispennis*, sp.n., fifth tarsal segment and claws of hind leg (♀); *c*—*P. marmoratus*, fourth and fifth tarsal segments and claws of hind leg (♀); *d*—*P. marmoratus*, fifth tarsal segment and claws (♂) (ventral view); *e*—*P. marmoratus*, tarsus of hind leg (♀). (*a* × 220 circa; *b*, *c*, *d* × 480; *e* × 125.)

laterally and posteriorly. Pleurae brown. Scutellum, yellowish-brown, dark brown medially, bearing, in both sexes, two ventral and two lateral marginal bristles. Post-scutellum dark brown, with two, relatively large, rounded, greyish or yellowish-brown spots anteriorly. *Wings* hyaline with two small, dark brown, spots, one covering the extremities of the costa and first and third veins, the other immediately before, and partially including, the anterior transverse vein. Venation in the female similar to that shown in

fig. 5; venation in the male, resembling that shown in fig. 7, but the tip of the costa less swollen and the radial cell smaller, its length not more than two-thirds the length of its petiole; anterior veins cream-coloured, bearing several, moderately stout, pale brown hairs, the wing membrane between them slightly infuscated. Decumbent hairs sparse, most numerous along the anterior margin from the extremity of the costa to the wing apex, and between the branches of the fourth vein, where they extend backwards almost as far as the middle of the cell; in the male, the decumbent hairs are extremely scanty, being limited almost to a single marginal row, extending from the costa to a little below the upper branch of the fourth vein. Halteres cream-coloured or whitish, the extreme bases of the knobs infuscated. *Legs* testaceous with dark brown bands: femora, each with a broad median and a narrower apical dark band; tibiae, each with three dark bands, at base, middle and apex, the middle band sometimes indistinct; tarsi often slightly infuscated distally, the first tarsal segments of the middle and hind legs more strongly infuscated, appearing almost black when held in certain positions. Fore coxae, femora and tibiae of male armed with one or more stout, black, spines: the fore coxae each with one; the fore femora each with a few at the apex, the middle femora each with a longitudinal row of five or six, and the hind femora each with a lateral row of six, a ventro-lateral row of three or four (smaller) and two apical; the fore and hind tibiae each with a lateral row of six to eight, and the middle tibiae each with one (large) near the base. Claws in the female (fig. 2 *c*), equal and simple, at least two-thirds the length of the fifth segment; in the male (fig. 2 *d*) bifid, shorter, about one-half the length of the fifth segment. *Abdomen* of female very dark brown, almost black, with white, or dusky-white, admedian and lateral markings as shown in fig. 4 *a*. In the male, the admedian pale spots are similar, but those on the second and third segments are larger, almost semi-circular, and less widely separated centrally, while those on the fifth to seventh segments meet in the middle line (at least in some specimens) and form transverse bands—the visible portions of these segments being almost entirely white; the form and arrangement of the lateral markings cannot be definitely observed in the dry, undistended, examples at our disposal. Spermathecae (fig. 9 *a*)

two in number, highly chitinised, sub-spherical (diameter 55μ) each with a long (38μ), chitinous, stalklike process arising below the duct and directed posteriorly; the duct chitinised for a moderately long distance (13μ) at its commencement.

HYPOPYGIUM (fig. 3). Dark brown, highly chitinised. *Ninth segment*: sternite with a shallow ventral excavation; tergite rather short, very slightly chitinised posteriorly, the dorsal surface with six long, strong, hairs, of which four are arranged in a transverse row on the distal third and two are situated laterally immediately above the bases of the forceps, the posterior margin gently rounded

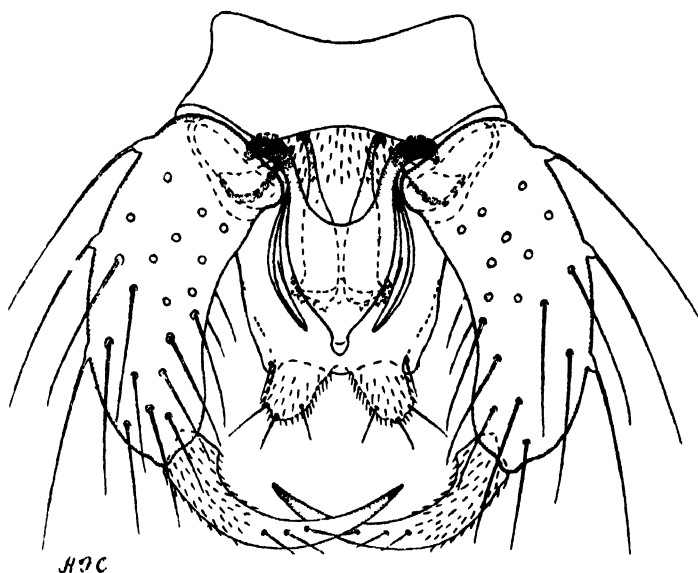


FIG. 3. *Prionognathus marmoratus*, sp.n., male hypopygium, ventral view. ($\times 400$ circa.)

centrally with the lateral finger-like processes reduced to small, rounded flanges, each bearing a short hair, the apical lobe-like processes relatively large. *Forceps*: side-pieces well developed and highly chitinised, rather sparsely clothed with long hairs; clasper long, highly chitinised, hook-like, tapering to a sharp point, the basal portion densely clothed with minute hairs. *Harpes* moderately large, the proximal portions narrowed and highly chitinised, the distal portions broad and flattened, especially at the tips, which are produced slightly on their outer sides. *Aedoeagus* somewhat

shield-shaped, with a chitinous, flange-like process on each side; distal extremity in the form of a short, more or less conical, beak-like process with the apex directed ventrally; limbs highly chitinated basally, with the proximal ends everted; ventral wall chitinated, the anterior half of the membrane connecting it with the ninth sternite studded with spicules.

HABITAT: Accra, Gold Coast; three males and six females, collected in the evening upon the windows of the laboratory, January to March, 1920. Bonny, Nigeria, three females, collected by Dr. H. E. Annett upon windows, 6.30 a.m., May 13th, 1900 (in collection of the Liverpool School of Tropical Medicine).

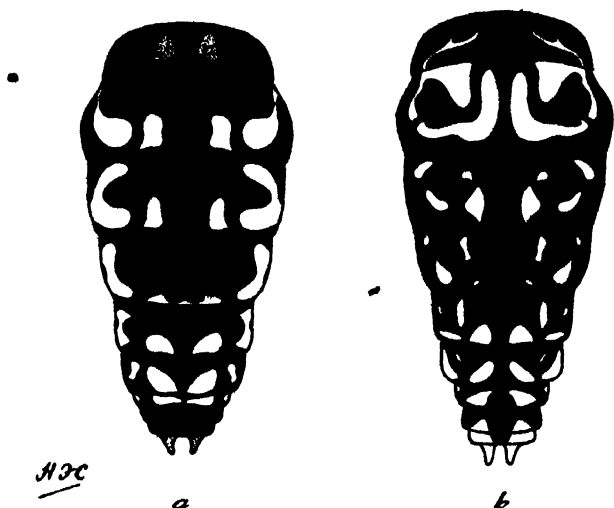


FIG. 4. Abdomens (distended) of females, showing ornamentation. *a*—*P. marmoratus*, sp.n., *b*—*P. maculipennis*, sp.n. (semi-diagrammatic).

Prionognathus maculipennis, sp. n.

MEASUREMENTS.

| | | | | | | | |
|--------------------------|-----|-----|-----|-----|-----|-----|---------|
| Length of body | ... | ... | ... | ... | ... | ... | 1.3 mm. |
| Length of wing | ... | ... | ... | ... | ... | ... | 1.1 mm. |
| Greatest breadth of wing | ... | ... | ... | ... | ... | ... | 0.4 mm. |

Head greyish-brown with brown hairs. Eyes narrowly separate. Clypeus and proboscis dark brown with brown hairs; mouth-parts similar to those of *P. marmoratus* but the labrum and hypopharynx broader distally, the former bluntly rounded at the apex with three or four closely apposed apical teeth on each side of

the middle line, and seven or eight broader, more rounded, lateral teeth on the distal third, the hair-like processes apparently absent. Palpi brown, the second to fifth segments sub-equal, the fifth swollen distally, with an apical whorl of five or six stout hairs. Antennae brown, the torus darker than the other segments, segments four to ten oval to sub-cylindrical, from about one and one-third to two and one-half times the greatest width. *Thorax* grey or greyish-brown with dark brown more or less V shaped marks covering the anterior pits, and small rounded, dark brown or blackish, spots at the bases of the hairs, which are brown and rather scanty. *Pleurae* dark brown. *Scutellum* greyish-brown, dark brown medially, bearing four central bristles—two anterior and two marginal, the latter close together. *Post-scutellum* dark brown, with two large pale grey, partially contiguous, areas anteriorly. *Wings* hyaline, the membrane between the thickened anterior veins slightly infuscated, with six small, irregularly shaped, blackish spots situated as shown in fig 5. Decumbent hairs confined to the

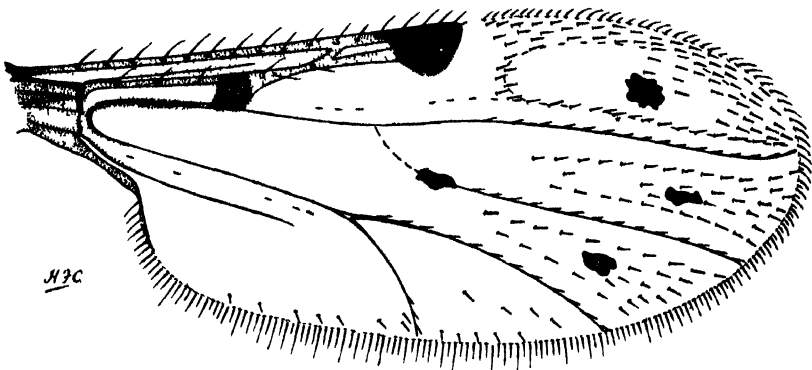


FIG 5 *P. maculispennis*, ♀ p n, wing of female (× 90 circa)

distal half of the wing, more numerous than in *P. marmoratus*, especially in the anterior region above the upper branch of the fourth vein, and between the branches of the fourth vein, where they extend backwards considerably beyond the middle of the cell. Halteres with greyish-brown knobs, and paler, yellowish-brown, stems. *Legs* brown, the tarsi, except the metatarsi of the hind legs, and bands (most conspicuous on the hind legs) on the femora and tibiae paler, yellowish-brown, femora each with a sub-apical band, the basal thirds of the hind pair also pale ventrally, tibiae

each with two bands, a sub-basal and a sub-apical. Claws (fig. 2 *b*) unequal, one very large, almost as long as the fifth segment, the other small, scarcely half as long as the segment. Abdomen dark brown, with pale grey or greyish-white markings as shown in fig. 4 *b*. Spermathecae (fig. 9 *b*) single, large (54μ by 81μ) highly chitinated, pear-shaped, the posterior third with minute, circular, less highly chitinated areas; the duct chitinated for a short distance at its commencement.

HABITAT: Accra, Gold Coast; a single female taken in the evening upon a window in the laboratory, March 25th, 1920.

Prionognathus pseudomaculipennis, sp. n.

MEASUREMENTS.

| | | | | | | | |
|--------------------------|-----|-----|-----|-----|-----|-----|----------|
| Length of body | ... | ... | ... | ... | ... | ... | 1.0 mm. |
| Length of wing | ... | ... | ... | ... | ... | ... | 0.85 mm. |
| Greatest breadth of wing | ... | ... | ... | ... | ... | ... | 0.3 mm. |

This species greatly resembles *P. maculipennis*, but is considerably smaller and differs also in the following characters.

Head: Eyes narrowly contiguous anteriorly. *Thorax*: Scutellum with two central marginal bristles only. *Wing* (fig. 6) with rather

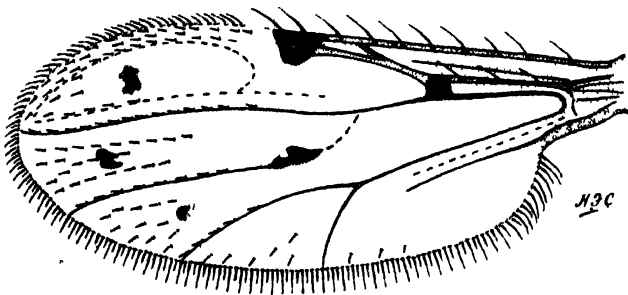


FIG. 6. *P. pseudomaculipennis*, sp.n., wing of female. ($\times 90$ circa.)

less numerous decumbent hairs, those between the branches of the fourth vein not, or scarcely, extending beyond the middle of the cell. *Abdomen* (as seen when undistended) with large yellowish or greyish-yellow pale areas; the admedian pale areas apparently more extensive, especially on segments three and four. Spermathecae (fig. 9 *c*) single, highly chitinated, sub-spherical (diameter 46μ); the chitinated portion of the duct short and broad.

HABITAT: Accra, Gold Coast; one female taken in the evening upon a window in the laboratory, February, 1920. Oblogo, Gold Coast; one female reared from rotten wood from canoes, May, 1920.

Prionognathus maculithorax, sp. n.

MEASUREMENTS.

| | | | | | | | |
|--------------------------|-----|-----|-----|-----|-----|-----|---------|
| Length of body | ... | ... | ... | ... | ... | ... | 1.1 mm. |
| Length of wing | ... | ... | ... | ... | ... | ... | 1.0 mm. |
| Greatest breadth of wing | ... | ... | ... | ... | ... | ... | 0.3 mm. |

Head brown. Eyes separate; the space between them wedge-shaped, broadest at the vertex. Clypeus and proboscis brown, with brown hairs. Palpi brown, the second to fifth segments sub-equal, the fifth swollen distally with an apical whorl of relatively stout hairs. Antennae greyish-brown, the torus darker than the other segments; terminal segments (thirteen to fifteen) elongate, the thirteenth distinctly larger than the others. *Thorax* pale greyish-brown, with small dark spots; the markings very similar to those of the two preceding species (*P. maculipennis* and *P. pseudo-maculipennis*), sparsely clothed with brown hairs. Pleurae and post-scutellum brown. Scutellum pale brown with two central marginal bristles. *Wings* hyaline with small blackish spots as shown in fig. 7. Decumbent hairs scanty, almost confined to the anterior

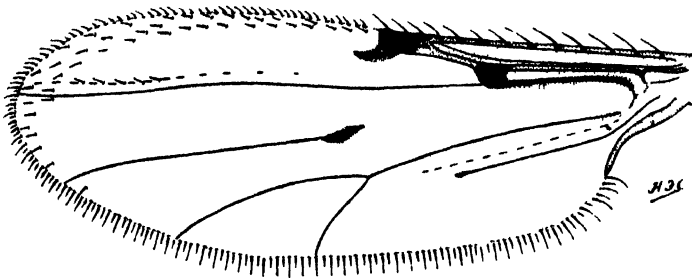


FIG. 7. *P. maculithorax*, sp. n., wing of male. ($\times 90$ circa.)

and distal margins. Halteres with brown knobs. *Legs* brown, with paler bands as in *P. maculipennis*; femora and tibiae without spines. Claws bifid, equal, about one-half the length of the fifth tarsal segment. *Abdomen* dark brown, with pale grey markings

somewhat similar (so far as can be determined from the single specimen obtained) to those in *P. maculipennis*.

HYPOPYGIUM (fig. 8). *Ninth segment*: tergite rather short, the posterior margin slightly curved and bearing, on each side, a long finger-like process; sternite relatively large, produced posteriorly, on each side of the middle line, into a rounded lobe covered with minute hairs. *Forceps*: side-pieces moderately chitinised, dark and hairy; claspers less chitinised, the basal third broad, pubescent; the distal extremity pointed, bearing a few small hairs. *Harpes* large, strongly chitinised; proximal portion directed laterally, forming almost a right angle with the distal portion; the latter broad

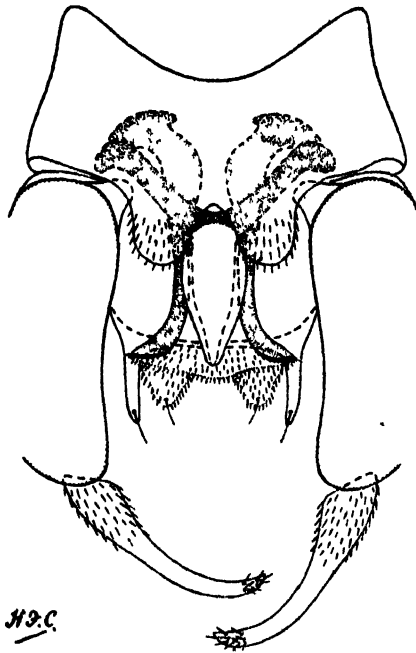


FIG. 8. *Prionognathus maculiborax*, sp.n., male hypopygium, ventral view.
($\times 400$ circa.)

at the base and curving inwards for a short distance, then extending almost directly backwards (becoming narrower near the middle) and terminating in a slightly expanded, outwardly directed extremity, the posterior margin of which bears a number of minute hairs. *Aedoeagus* large; the anterior portion (limbs) very strongly chitinised, and occupying a more ventral position than the rest, the

posterior portion relatively broad, but narrowing in the distal fourth and ending in a bluntly rounded apex.

HABITAT: Accra, Gold Coast, one male taken in the evening upon a window of the laboratory, January, 1920.

Genus *ATRICHOPOGON*, Kieff.

Ceratopogon, Meig. (pro parte). Illiger's Mag. Ins. Vol. II, 1803.

Atrichopogon, Kieff. Ann. Soc. Scient. Brux. Vol. XXX, 1906.

Ceratopogon, Mall. (nec. (Meig.), Edw.). Bull. Ill. Sta. Lab. Nat. Hist. Vol. X. Art. vi, p. 304, 1915.

Malloch's restriction of the name *Ceratopogon* to the group of species exemplified by *fusculus*, Coq (the species first cited by him) cannot be maintained. *C. communis*, Meig, the accepted type of *Ceratopogon*, was thought by Johannsen (1908) and others, including Malloch, to belong to the same group as *evilis*, Coq, which by Coquillett's (1910) designation is the type of *Atrichopogon*, Kieff. The adoption of this use of the name *Ceratopogon*, i.e., replacing *Atrichopogon*, seemed probable (even although Kieffer (1917 and 1919), by unjustifiably applying *Ceratopogon* to *Forcipomyia*, rejected it), until Edwards (1920) showed conclusively that *C. communis* not only differed in structure from the *evilis* type, but that it did not belong to any of Kieffer's genera.

The genus *Atrichopogon* includes those midges in which the eyes are bare, the metatarsi of the hind legs considerably longer than the second segments, the empodia well-developed, and the wings bear long hairs (usually somewhat scanty and confined to the distal portion) and possess a long and narrow second radial cell which extends to the distal third. Two females, of a single species, only were obtained in this investigation, and no detailed account of the external morphology can therefore be given. As, apparently, no attention has been paid to the mouth-parts of the females of this genus, a short description of those of our specimens may be of interest.

Mouth-parts. The proboscis of the female is slightly shorter than the height of the head. The labium is soft and similar to that of *Culicoides* (vide Carter, Ingram and Macfie, 1920), but possesses longer hairs distally. The labrum is relatively broad, but the

distal third tapers to a pointed, or very narrowly rounded, apex, and is fringed on each side with numerous, short, hair-like processes. The hypopharynx is less strongly chitinised than the labrum; the proximal two-thirds are broad, the distal third attenuated, scoop-like, and without teeth or lateral processes. The mandibles are large, pointed, strongly chitinised structures, each with from eighteen to twenty teeth on the inner distal margin; the ten or twelve anterior teeth are larger and stronger than the others. The maxillae are much less strongly developed than the mandibles; they are not more than three-quarters the length of the latter, are slightly chitinised and obliquely truncate distally, the inner distal edge bearing eight inconspicuous, somewhat rounded, teeth.

Atrichopogon xanthoaspidium, sp. n.

MEASUREMENTS.

| | | | | | | | |
|--------------------------|-----|-----|-----|-----|-----|-----|---------|
| Length of body | ... | ... | ... | ... | ... | ... | 1.7 mm. |
| Length of wing | ... | ... | ... | ... | ... | ... | 1.3 mm. |
| Greatest breadth of wing | ... | ... | ... | ... | ... | ... | 0.5 mm. |

Head brown, clothed with brown hairs. Eyes broadly contiguous above. Clypeus, palpi and proboscis dark brown, with dark brown hairs, first palpal segment small but distinct, second, fourth and fifth segments sub-equal, third segment at least one and one-half times the length of any of the others, slightly inflated at the distal third where a small, but deep, sensory cup exists. Antennae dark brown, with slightly paler hairs; segments four to ten short and broad, the tenth sub-spherical, the others broader than long, the length varying from half to two-thirds the breadth; last five segments (eleven to fifteen) elongate, from two and a half to three and a half times as long as broad, their combined lengths almost twice those of segments three to ten, the fifteenth terminating in a small stylet. *Thorax* dark brown, pruinose, showing indistinct yellowish-brown, sub-circular, anterior areas when held in certain positions; clothed with short golden-brown hairs. *Pleurae* dark brown. *Scutellum* yellowish, bearing two sub-median and two lateral bristles and several (fifteen to twenty) short hairs. *Post-scutellum* dark brown. *Wings* clear, unspotted, the anterior veins

yellowish-brown, venation and arrangement of decumbent hairs, as in fig. 10. Halteres with white knobs and infuscated stems. *Legs* almost uniformly yellowish-brown, but with indications of darker knee-spots, and with the tarsal segments slightly infuscated. Claws equal, about half the length of the fifth tarsal segment, each with a very minute, sub-apical, tooth. Empodium well-developed, at least as long as the claws. *Abdomen* brown, rather darker than

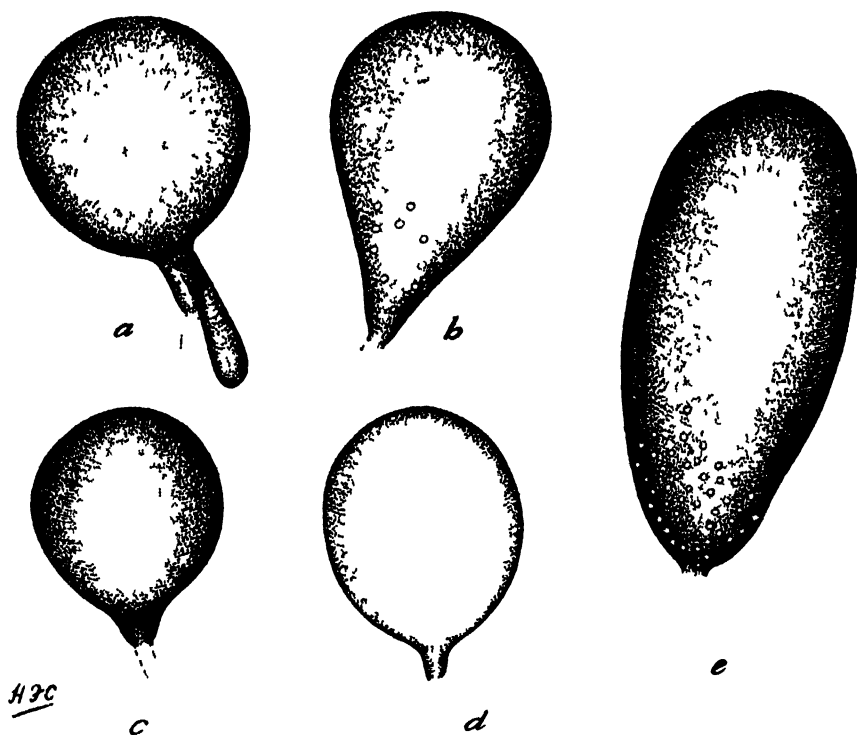


FIG 9 Spermathecae of a—*Prionognathus marmoratus*, sp n (one only shown), b—*P. maculipennis*, sp n, c—*P. pseudomaculipennis*, sp n, d—*Stilobezzia spirogyrae*, sp n, e—*Atricopogon xanthoaspisidum*, sp n ($\times 540$ circa)

the thorax, ventral surface paler. Spermathecae (fig 9 e) single, large (length 120μ , greatest breadth 49μ), sac-like, heavily chitinated, the posterior third narrower, with the chitin strongly pitted, appearing as if covered with minute white dots, no portion of the duct chitinated.

HABITAT Accra, Gold Coast. Two females, collected in the evening upon a window in the laboratory, May, 1920

This species is the third of the genus *Atrichopogon* to be described from the Ethiopian region; the others are *A. anemotis*, Kieff., and *A. tropicus*, Kieff., both of which were described by Kieffer in 1913. From *A. anemotis*, of which the male only is known, it apparently differs in size, colouration of the abdomen, and

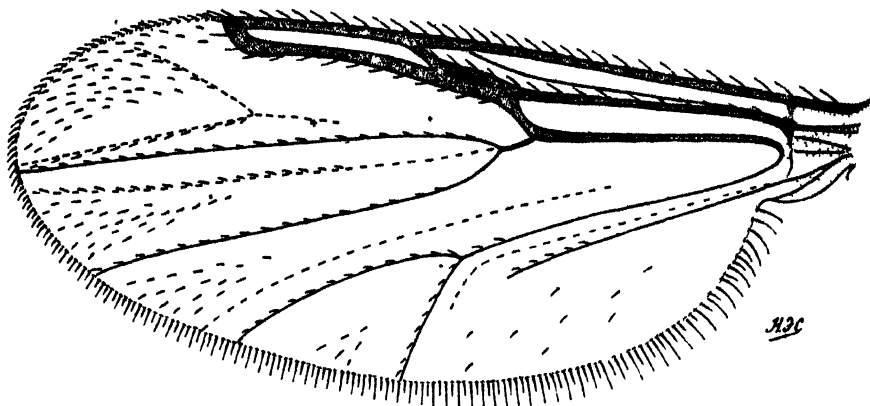


FIG. 10. *Atrichopogon xanthoaspidum*, sp.n., wing of female. ($\times 90$ circa)

wing venation, and from *A. tropicus* in venation and arrangement of the larger wing hairs. Judging by Kieffer's figure, the petiolate portion of the fourth vein in *A. anemotis* is almost twice the length of that of *A. xanthoaspidum*, while in *A. tropicus* bifurcation takes place at the cross vein.

Genus *STILOBEZZIA*, Kieff.

Stilobezzia, Kieff. Rec. Ind. Mus. Vol. VI, p. 118, 1911.

Hartomyia, Mall. Bull. Ill. Sta. Lab. Vol. X. Art. vi, p. 339, 1915.

This genus was erected by Kieffer for species of *Palpomyia* and *Johannsenomyia* in which the fork of the fourth longitudinal vein is petiolate. The chief generic characters, according to this author (1919) are—wings glabrous, the first and third veins forming two radial cells of which the second is longer than the first, the fourth vein petiolate, the fourth tarsal segment cordiform in both sexes, and the claws long, simple and very unequal in the female, short and equal in the male.

Kieffer's statement that the wings are glabrous is evidently not

intended to include microtrichia; these cover the whole surface, and are mentioned by him in his description of the type species (*S. festiva*). In the species described below the claws of the female are referred to as 'single with a large basal tooth,' as actually they are; but this statement does not imply any disagreement with the generic definition, since it is abundantly clear that, in our species, fusion has taken place and that the basal tooth represents the smaller claw.*

The larvae and pupae greatly resemble those of *Culicoides*. The larvae are vermiform with relatively short, strongly chitinated heads, and feebly-developed body hairs; the hypopharynx, however, is much more complex in structure, and is produced posteriorly into a relatively large hemispherical sclerite. The pupae differ from *Culicoides* (but agree with *Dasyhelea*) mainly in the greater size of the second abdominal segment.

Stilobezzia spirogyrae, sp. n.

| MEASUREMENTS. | Male. | Female. |
|---------------------------------|---------|---------|
| Length of body | 2.4 mm. | 2.0 mm. |
| Length of wing | 1.6 mm. | 1.7 mm. |
| Greatest breadth of wing | 0.5 mm. | 0.6 mm. |

Head: occiput green with brown hairs. Eyes, in the female contiguous, in the male narrowly separate. Clypeus and proboscis brown, with brown hairs. Palpi dark brown, slender, the first segment minute, the second and fourth short, the third and fifth considerably longer; third segment slightly inflated with a shallow, anterior depression from which arise several long, slender, sensory hairs. *Antennae*: in the female yellowish-brown, the torus darker with brown hairs, third to tenth segments sub-cylindrical, the third about four times as long as wide, the fourth to tenth from two and one-half to three and one-half times as long as wide; segments eleven to fifteen very slender, cylindrical from about seven to eighteen times as long as wide, increasing progressively in length to the last, which is about one and a half times as long as any of the others. In the male, rather paler brown with dark brown plumes; segments three

* In this connection it is interesting to note that with those species of *Pronognathus* in which the claws are unequal in size, the base of the smaller one rests upon, and appears to arise from, that of the larger.

to twelve short and broad, segments thirteen to fifteen greatly elongated, the fifteenth excessively long and thin. *Thorax*: dorsum in the female, shining greyish-green, becoming somewhat greenish pruinose anteriorly, with conspicuous sepia-coloured markings as follows: a broad median stripe extending from the anterior margin almost to the centre, two admedian stripes, broad on the anterior third, linear throughout the rest of their course, extending from the anterior margin to the scutellum, and two short lateral stripes each of which, commencing near the centre, extends anteriorly and then curves sharply inwards to join the admedian stripe near the middle of the broad portion; clothed with rather stout, brown hairs or bristles on the disc (arranged in median and admedian longitudinal rows) and sides. In the male the colouration of the dorsum resembles that of the female, but is more pruinose, especially laterally and in the region of the posterior depression. Pleurae greenish-pruinose, with a relatively large, dark brown patch over the coxae of the middle legs. Scutellum with seven strong brown bristles—one central marginal (usually absent in the male), two central sub-marginal, two central median and two lateral. Post-scutellum olive green. *Wings* pale grey, strongly iridescent, with dark brown markings and venation as shown in fig. 11. Halteres pale, the distal half of

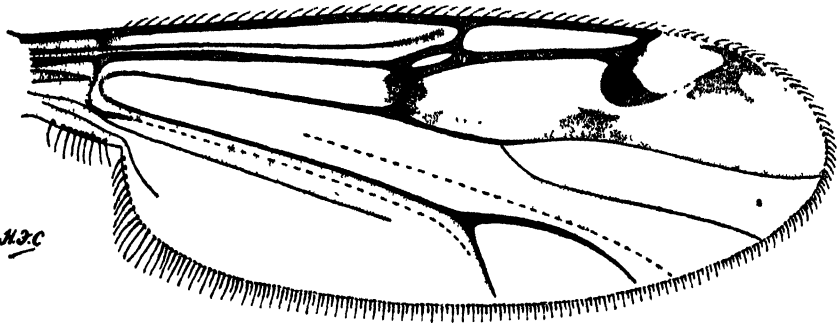


FIG. 11. *S. spirogyrae*, sp.n., wing of female. ($\times 65$ circa.)

the knobs dark brown. *Legs* (fig. 13) in both sexes pale olive green with olive brown markings; some of the tarsal segments armed with conspicuous, stout, black spines, and the first and second tarsal segments of all the legs bearing rows of small spines. Fore femora pale, slightly darker at the bases, the distal half of the middle femora and the whole of the hind femora (except the extreme apices)

infuscated. Tibiae similar to the femora but with the distal extremities dark. Tarsi with the last two segments slightly darkened; in both sexes, the small spines on the first and second segments of the fore and middle legs are arranged in a single ventral, or ventro-lateral, longitudinal row, while on the hind legs there are two ventro-lateral rows on each of these segments and, in addition, on the first segment a lateral row extending from the base to about the middle; five large, black spines are present on the middle tarsi—one at the base of the first segment and a pair at the apices of the first and second segments—and, in the female, an additional pair occurs on the fifth segment of the fore and middle legs. Claws in the female single, almost as long as the fifth tarsal segment, with a large basal tooth; in the male less than half the length of the fifth segment, equal, bifid at the tips. *Abdomen* pale green with dark brown markings, which are most conspicuous on the posterior segments; clothed with brown hairs and bearing, near the centre of each of the second to fifth tergites, a tuft of relatively large, black bristles. Spermathecae (fig. 9d) two in number, slightly chitinated, sub-spherical (57μ by 48μ); the commencement of the duct chitinated for a short distance.

HYPOPYGIUM (fig. 12). *Ninth segment*: sternite with a relatively shallow ventral excavation; tergite short, the posterior margin irregularly rounded without lateral finger-like processes. *Forceps*: side pieces relatively large, each with a broad, inwardly projecting basal process; the proximal portion very broad, narrowing sharply near the middle, the distal portion tapering gradually to a broadly rounded apex. Claspers stout, terminating in a large, stout, blunt process; clothed with minute hairs, intermixed with several strong hairs on the basal half and three or four delicate hairs near the apex. *Harpes* slender, very heavily chitinated; the proximal portion short (about one-fourth the entire length) expanding anteriorly, the distal portion broad anteriorly, gradually tapering and directed inwards and downwards to about the apical third, then extending in an antero-posterior direction to the apex, which is swollen and hooked. *Aedoeagus* somewhat V-shaped, less heavily chitinated than the harpes, with the distal extremity expanded into a thinly chitinated, flange-like structure and directed dorsally at almost a right angle to the plane of the ventral wall; in ventral view the distal portion

frequently appears to be divided centrally into two separate flanges. Ventral wall membranous, continuous with the membrane connecting it with the ninth segment; spicules absent.

PUPA. Length 2.9 mm. to 3.4 mm., average of four 3.2 mm. *Respiratory trumpets* as shown in fig. 13 g, length 0.35 mm.; they are rather slender, dark-tipped structures, sharply constricted at the base and slightly narrowed near the distal fourth; the main tracheal

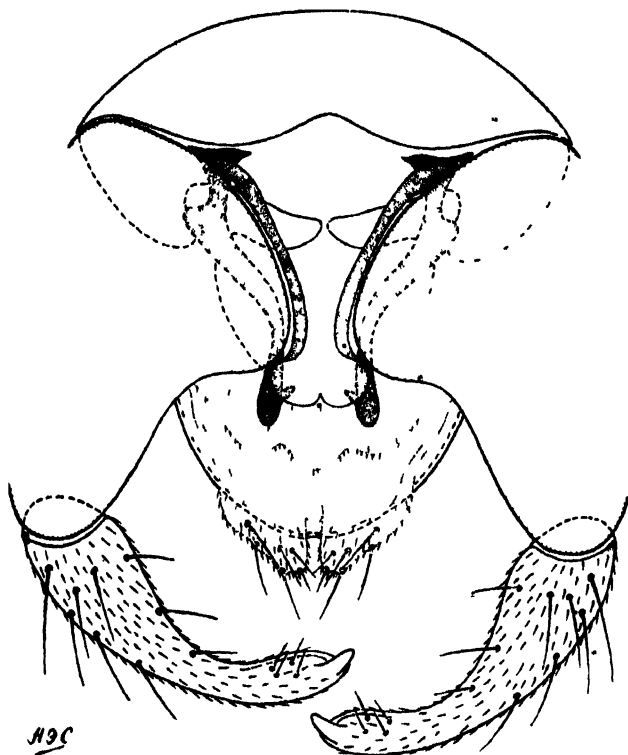


FIG 12. *S. spirogyrae*, sp.n, male hypopygium, ventral view. ($\times 400$ circa)

trunk is relatively broad, devoid of lateral branches, and terminates in a number (about fifteen) of short, blunt processes which lead to the surface and are arranged as indicated in the figure. *Cephalothorax* somewhat infuscated dorsally. Anterior marginal tubercle, large, conical, bearing a short, stout spine; anterior dorsal, small with a short spine and a hair; anterior dorso-median,* very small,

* This tubercle is not present in *Culicoides*, it is situated on the inner side and just above the base of the trumpets.

bearing a short spine and a hair; anterior dorso-lateral, small, bearing a long, terminal hair, and a short, lateral bristle; ventro-lateral, represented by three small hairs; ventro-median, absent. Dorsal tubercles reduced to two pairs of minute bristles or hairs.

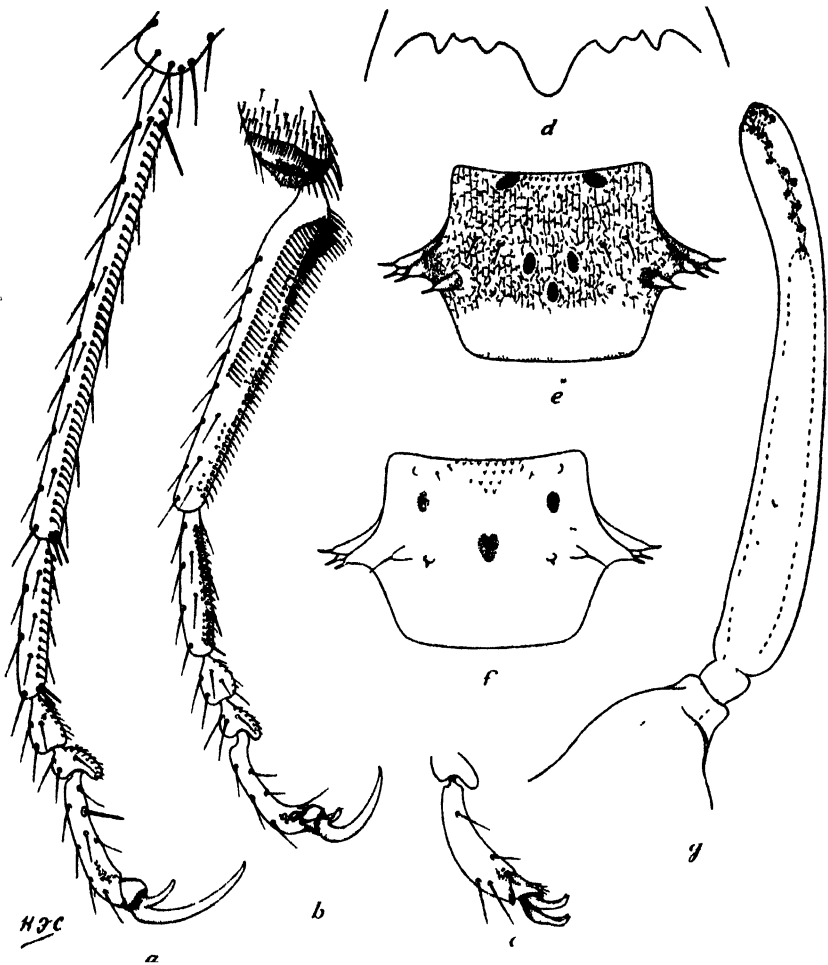


FIG. 13. *Stilobezzia spirogyrae*, sp.n. a—tarsus of middle leg (♀); b—tarsus of hind leg (♀); c—fifth tarsal segment and claws of middle leg (♂); d—teeth on posterior margin of hypopharyngeal sclerite of larva; e—fifth abdominal segment of pupa (dorsal view); f—fifth abdominal segment of pupa (ventral view—surface marking, other than pigmented areas, omitted); g—respiratory trumpet of pupa. (a and b $\times 125$ circa, c $\times 260$; d $\times 1050$; e and f $\times 80$; g $\times 220$.)

Posterior dorsal tubercle small, bearing a short hair. *Abdomen*: first segment small and narrow, second segment large and broad, the others decreasing progressively in breadth towards the apex;

integument reticulated except on small areas (shagreened) at the bases of the last four or five segments, on the distal portions of the segments, and on small oval pigmented areas situated on each segment as shown in fig. 13 *e* and *f*. Anal segment with acutely pointed, widely divergent (directed almost at a right angle to the axis of the body), dark-tipped processes which are almost two-thirds the length of the segment. Dorsal tubercles: antero-submarginal, small, each bearing a short hair; postero-marginal,* the inner very small (larger on the eighth segment), bearing a minute hair, the outer large, with a short, stout spine. Ventro-lateral tubercles: antero-submarginal absent; postero-marginal, very large, each bearing a short, stout spine. Ventral tubercles: the inner minute, bearing a minute spine, the outer large, bearing a long hair.

LARVA. Length 4.5 mm. to 5.6 mm., average of five 5.1 mm. Greatest breadth 0.27 mm. to 0.37 mm., average of four 0.3 mm. *Head*: yellowish-brown, length about 0.3 mm., greatest breadth 0.2 mm. Median dorsal plate (clypeus) broad posteriorly. Eyes black, bilobed or reniform, the anterior portion small. Hairs very small, apparently arranged as follows: on the dorsal surface, one pair anterior admedian, one pair anterior dorso-lateral, two pairs central dorso-lateral, two pairs posterior dorso-lateral, and two pairs posterior admedian; on the ventral surface, two pairs anterior admedian, three central ventro-lateral, and one pair sub-central, admedian. Mental plate delicate, relatively short and broad, apparently without teeth. Hypopharynx strongly chitinated, the posterior margin, or what appears to correspond with the posterior margin in *Culicoides*, armed with a large, rounded, central tooth, and three small teeth or processes on each side (*c.f.* fig. 13 *d*). Mandibles simple, hook-like. *Body* with scanty and very minute hairs; those on the distal portion of the anal segment larger and stronger and arranged in six pairs, of which two pairs are smaller and more delicate than the others.

HABITAT: Oblogo, one male and one female bred from pupae obtained from a washing place in the river; Nsawam, four males

* The number and arrangement of the postero-marginal tubercles differ from those of *Culicoides*. In *Stilobessia* seven only are present—two dorsal, three ventro-lateral and two ventral; they are situated near the middle transverse line and form an almost continuous semi-circular lateral band—the innermost of the dorsal and ventral tubercles being situated a considerable distance from the middle line.

and three females reared from larvae and pupae taken from a swamp, 26th May, 1920.

This strikingly coloured midge is apparently the first species of *Stilobezzia* to be described from Tropical Africa. The larvae and pupae of the Nsawam examples, referred to above, were associated with a species of *Spirogyra*. A specimen of this alga was very kindly examined for us by Miss Nellie Carter, of Birmingham University, who wrote as follows:—‘The alga is a species of *Spirogyra*, Link, but it is sterile, and in the absence of ripe zygospores it is quite impossible to name the species. There is also amongst it a little *Oscillataria tenuis*, Ag. (*Cyanophyceae*), and a quantity of the filamentous diatom *Fragillaria virescens*, Ralf, but the *Spirogyra* is present in by far the greatest bulk.’

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MUSCA INFERIOR, STEIN, TYPE OF A NEW GENUS OF PHILAEMATOMYINE FLIES (*DIPTERA*)

BY

PROFESSOR M. BEZZI

*Turin, Italy**(Received for publication 22 November, 1920)*

In 1909, Stein, the well-known Monographer of the Anthomyidae of the world, described a *Musca inferior* from Java, pointing out as the main characters, the greater size, yellowish colour of palpi, separated eyes of the male, presence of only a single pair of well-developed dorso-ventral macrochaetae, and the lineate pattern of the abdomen. These characters are sufficient for the immediate recognition of the fly.

In 1912, Patton and Cragg published in these *Annals* a short preliminary description of an Indian fly under the name of *Philaematomyia gurnei*, of which, later in the same year, they gave a detailed description with beautiful figures.

In 1916, in my second paper on the Philippine *Diptera*, I established the synonymy of the two above-named species, leaving them in the genus *Philaematomyia*, Austen, under the name of *Ph. inferior*, Stein, and establishing their presence in the Philippines.

Subsequently, in 1918, two papers appeared with more notices on this same fly, but without referring to the synonymy given by me. The first of these papers is by Stein, who, recording the species, under the generic name of *Musca* from Formosa, points out its affinity with *Crassirostris insignis* (the type species of the genus *Philaematomyia*), and recognises that the two species must be placed in one new genus; on this occasion he adds to the original description the important characters of the form of the proboscis and of the bristles of the third longitudinal vein of the wings. The second paper is by Awati, who, in describing a new species of *Philaematomyia* from India, made a critical review of the genus,

dealing with the microscopical genital armature of *gurnei* in relation to that of the three other species of the genus.

Finally, in 1919, Stein (*b*, p. 40, and *c*, p. 47) repeated the characters and definitely placed his *Musca inferior* with his *Musca crassirostris* in the same group *Philaematomyia*, which, however, is not adopted as a distinct genus in the general catalogue of the exotic Anthomyidae (*a*, pp. 103-105).

I have, in my private collection, much very fine material of *Musca*, s.l., and of *Philaematomyia*, s.l., from Africa, Asia and Australia; and, moreover, I have at present in my hands the fine collection of these flies from the Indian Museum, Calcutta. I am thus now in a position to complete my attempt of 1911 (*b*, pp. 82-98) to distinguish the species of this important and difficult group on macroscopical characters only.

I am aware of the microscopical characters employed by Awati (1916, *a*) for the distinction of the Indian species, and I realise their importance in the exact specific determination in such a uniform group of insects as that of *Musca* and related forms. But I am always of the opinion that external macroscopical characters are, for practical purposes, more useful for quick determination. A number of these characters have already been used by Awati himself, who has also found some new ones (1916, *b*), such as tympanic bristles or hairs, hypopleural hairs, position of antennal grooves, etc., and others can always be discovered.

Thus, in examining my specimens of *inferior* (*gurnei*) I have found that they possess an important and easily visible character hitherto overlooked by all the writers, although well shown in the figures given by Patton and Cragg (1913, *a*, Pl. VII, and 1913, *c*, Pl. XLVII). I ascribe to this character such an importance that I think that it is sufficient for the erection of a new genus for the above-named species. In the higher and middle Myiadaria this is a matter of opinion; but it is true that all the main workers in these groups, beginning with Robineau-Desvoidy in 1830, following with Rondani and Brauer to Villeneuve and Tyler-Townsend to-day, have always multiplied the genera.

Thus, while Stein, even in his most recent works on the Anthomyidae (1916 and 1919, *a*), has recognised only one great genus *Musca*, other writers have divided these forms into two sub-

families (*Philaematomyiinae* and *Muscinae*), with no less than seven genera; *Philaematomyia*, *Pristirrhynchomyia*, *Viviparomusca*, *Musca* (*Promusca*), *Eumusca*, *Placosayia*, *Plexemya* and *Biomyia* (*Biomya*), and what, by Professor Stein, is considered to be only one species, under the name of *Musca corvina* (now better known as *autumnalis*), is referred to by others as two distinct species belonging even to two different genera, *Viviparomusca larvipara*, Ports. (*Corvinoides*, Seh. and Dziedz.) and *Eumusca autumnalis*, Deg. (*corvina*, Fabr., *ovipara*, Ports.).

The character on which I erect the new genus is to be found on the calypters, the lower or thoracic squama of which bears, in *Ph. inferior* (*gurnet*), a number of dark hairs, easily visible with the aid of an ordinary lens; the hairs are placed on the inner part of the upper surface of the organ. This character is of great value in the classification of other flies, chiefly of the *Calliphorinae*; and, moreover, it is unique among the forms at present known in the groups *Philaematomyia* and *Musca*, s.l.

Ptilolepis, nov. gen.

Geno-type: *Musca inferior*, Stein, 1909.

Differing from all known genera, or sub-genera, of its group, in having the lower squama of the calypters hairy on its upper surface. The following additional characters of the genus must be recorded: Eyes bare, rather distant in the male. Parafrontal hairs of the female arranged in more than one row. Outer vertical bristles not developed in the male. Ocellar plate with many hairs and bristles. Facial ridges ciliated inferiorly. Proboscis thickened basally, with chitinous terminal teeth. Thorax with only one pair of strong post-sutural dorso-central bristles, before them another pair of much smaller ones; praesutural dorsocentral bristles entirely wanting; tympanic bristles present; no hypopleural hairs. One pair only of discal scutellar bristles, the anterior ones being much the smaller. Wings with the basal portion of the radius setose (with one bristle only), and with the third longitudinal vein setose on its whole length below (that is, on the under surface of the wing). Fourth longitudinal vein with a rounded, but deep, terminal bend, after which the apical cross-vein is concave outwardly. •

To show the position of the new genus in relation to the other

known forms of *Philaematomyia* flies, the following table may be given:—

1 (2). Lower squama of calypters hairy above; tympanic bristles present, ocellar triangle bristly; facial bristles ascending; palpi of a yellowish colour; no distinct praesutural dorsocentral bristles; third longitudinal vein setose on the whole length below; abdomen with three longitudinal black stripes. *Ptilolepis inferior*, Stein.

2 (1). Lower squama of calypters quite bare; no tympanic bristles or hairs; ocellar triangle not or less bristly; facial bristles not ascending; praesutural dorsocentral bristles short but distinct; third longitudinal vein quite bare, or exceptionally with one to two bristles only near its extreme base; abdomen with not more than one black longitudinal stripe.

3 (4). Basal bulb of the proboscis very much thickened; palpi yellowish; para-frontal hairs of the female in more than one row, acrostichal hairs near the suture disposed in eight or more irregular rows; abdomen of the male grey, destitute of shimmering areas, with a black discontinuous longitudinal stripe. *Philaematomyia crassirostris*, Stein.

4 (3). Proboscis not so thickened at base; palpi black; para-frontal hairs of the female in one row; acrostichal hairs in no more than four to six rows; abdomen of the male yellowish, with shimmering areas sometimes not very well developed.

5 (6). First abdominal segment of the male (Awati's second segment) entirely black; black longitudinal median stripe of abdomen continuous; smaller species 4 to 5 mm. in length, with oviparous female as in the preceding species. *Pristirrhynchomyia lineata*, Brunetti.

6 (5). First (Awati's second) abdominal segment of the male yellowish with black base and median stripes; black longitudinal stripe discontinuous, species of greater size, 5 to 6 mm. long, with larviparous female. *Pristirrhynchomyia indica*, Awati.

CATALOGUE OF THE SPECIES.

I. *Ptilolepis inferior*, Stein.

Musca inferior, Stein (1909), p. 213; (1918) p. 149, (1919, *a*) p. 104; (1919, *b*) p. 40; (1919, *c*) p. 47; De Meijere (1918), p. 321.

Philaematomyia gurnei, Patton and Cragg (1912, *a*), p. 513; (1913, *a*) p. 28, Pl. VII, (1913, *c*) p. 356, Pl. XLVII, fig. 2; Cragg (1913), p. 23, Pl. V; Castellani and Chalmers (1913), p. 709; Awati (1918), p. 538, Pl. L, LI and LIV.

Philaematomyia inferior, Bezzi (1916), p. 29; (1917) p. 130; Banks (1919), p. 187.

In addition to the known localities of Java, Sumatra, India, Formosa and Philippines, I have the species from Annam, and, moreover, I have in my collection types which enable me to confirm the above synonymy. The present species, like the others, is found only on cattle; it was recorded without a name by Mitzmain (1912), p. 498 and (1913) p. 41, together with *crassirostris* and other flies, in his work on *Stomoxys calcitrans* and its bionomics in the Philippines.

II. *Philaematomyia crassirostris*, Stein.

Musca crassirostris, Stein (1903), p. 646; (1918) p. 148; (1919, *a*) p. 103; (1919, *c*) p. 47, Bezzi (1911, *a*), p. 117; De Meijere (1918), p. 321.

Philaematomyia insignis, Austen (1909, *a*), p. 298, figs. 1-3; (1909, *b*) p. 137, figs 1-3; Howlett (1909), p. 646, Pl. LXX; Brunetti (1910), p. 90, Pl. VIII; Alcock (1911), p. 162, fig. 62; Bezzi (1911, *a*), p. 117; Geddoelst (1911), p. 228; Cragg (1912), pp. 1-17, Pl. I-V; Patton and Cragg (1912), pp. 515-520, figs. 1-4; Surcouf and Gonzalez-Rincones (1912), p. 160, fig. 91; Brunetti (1913), p. 43; Castellani and Chalmers (1913), p. 709, Cragg (1913), p. 26, Pl. I; Patton and Cragg (1913, *a*), p. 26, Pl. VIII; (1913, *b*) p. 13; (1913, *c*) p. 357, Pl. XLVII; Cornwall and Patton (1914), p. 569; Fletcher (1916), p. 78; (1917) p. 91; Awati (1918), p. 539, Pl. L, LII and LIV.

Philaematomyia crassirostris, Bezzi (1911, *b*), pp. 88 and 98; (1916) p. 29; (1917) p. 130; Banks (1919), p. 187.

This species is common on cattle in tropical or sub-tropical

countries of Africa and Asia. It is recorded from Senegal, Congo Free State, Soḱotra, India, Ceylon, Sumatra, Java, Borneo, Formosa and Philippines. I have also received specimens from Canton, South China, collected by Professor C. W. Howard.

In the Mediterranean sub-region, the species is known from Cyprus (Austen) and from Galilee (Brunetti), Becker has found it in Egypt at Cairo, Luxor and Assuan; but I have at present no records from North Africa or from South Europe.

The synonym was first proposed by me in 1911 and recorded by Patton and Cragg in 1913; I have since sent typical Indian specimens of *insignis* to Professor Stein, who has compared them with the types of *crassirostris* and has found them identical.

III. *Pristirrhynchomyia lineata*, Brunetti.

Pristirrhynchomyia lineata, Brunetti (1910), p. 91, figs. 1-2 and Pl. VIII; Geddoelst (1911), p. 229; Surcouf and Gonzalez-Rincones (1912), p. 161, fig. 92.

Philaematomyia lineata, Patton and Cragg (1912, a), p. 509, Pl. XXV; (1913, a) p. 27, Pl. VI; (1913, c) p. 356; Castellani and Chalmers (1913), p. 709; Cragg (1913), p. 20, Pl. IV; Awati (1918), p. 539, Pl. L. and LIV.

Known only from India at present; but Professor Stein (1909, p. 212) has already described it from Java without a name as a variety of *Musca pollinosa*, Stein. This last species, of course, is a true *Eumusca*, which I have seen from Annam.

The species, of which I have seen a co-type in the Indian Museum collection, does not seem to be rare; I have before me numerous specimens from S. India, Trichinopoly (*Cajus*), and some others from S. China, Canton (Professor C. W. Howard).

IV. *Pristirrhynchomyia indica*, Awati.

Musca indica, Awati (1916), p. 138.

Philaematomyia indica, Awati (1918), p. 529, Pl. L-LV.

I have before me a male specimen of what I believe to be the present species; it was caught in Southern India, Trichinopoly (*Cajus*).

I have here placed the species in *Pristirrhynchomyia*; but the larviparous habit of the female, unique in the tribe, shows that the

present species is not congeneric with the others, as indicated also by the reproductive characters and by the more developed praesutural dorsocentral bristles. In this case a new genus must be erected for it, and all the four known genera of Philaematomyine flies will become monotypic.

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NOTE

From the above Bibliography it is interesting to remark how récent our knowledge of the *Philaematomyine* flies is, being all subsequent to 1900. The dates of description of the four known species are 1903, 1909, 1910 and 1916; no doubt additional forms still remain to be discovered, but it seems that they must, in all probability, be only a few in number.

SOME STATISTICS OF FILARIASIS

BY

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Manson-Bahr's valuable monograph on Filariasis (1912) contains data which enable us to ascertain the microfilaria rate among persons with signs of 'filarial' disease, and, conversely, the disease rate among those infected with microfilaria. The value of these data lies in the fact that the author also gives control data, which similarly enable us to ascertain the respective rates among those without signs of disease and among those without microfilaria. It is exceptional to find such data in the literature, and almost always there is an absence of control observations. We may find recorded, for example, the microfilaria rate of a number of elephantiasis cases without any other information; this record by itself has little or no value, for if elephantiasis had no connection with filaria we should still expect such cases to show microfilaria if they formed part of a population infected with microfilaria.

Manson-Bahr presents his data in a summarised form in several tables. On studying these, several questions arose on which it seemed important to get further information, but it was not possible to do so from the summaries. Having written to Dr. Manson-Bahr on the matter, he very kindly placed at my disposal his note-books containing the protocols which his tables summarise, and I must express here my gratitude for his generosity in so doing. From the protocols I have compiled the tables given in this paper. Manson-Bahr's tables summarise observations made on 949 people; and in the protocols I have been able to trace 944 of these. For the form in which I have analysed these data I am alone responsible, and no blame attaches to Dr. Manson-Bahr for any errors on my part.

Our knowledge of the number and distribution of adult worms (*Filaria bancrofti*) in the body, and of the way in which they cause (*ex hypothesi*) the various lesions of the lymphatic system embraced in the term 'filarial disease,' is incomplete. Consequently authors have sought to strengthen the evidence for the association of adult worms with filarial disease by the use of evidence based on the association of embryos in the blood with various signs of the disease.

That it is not an easy matter to interpret such evidence the following considerations may shew:—

- (1) The *presence* of microfilaria in the blood may indicate
 - (a) the presence of a mature living female worm or worms in the body or
 - (b) the previous existence of such worms (now dead), because microfilaria, for all we know to the contrary, may live in the blood for an indefinite period.
- (2) The *absence* of microfilaria in the blood may indicate the absence of adult worms in the body, but not necessarily so, as living females have been found post-mortem, the presence of which was not revealed by microfilaria in the blood during life.
- (3) The *presence* of signs of filarial disease may indicate (a) the presence of a living worm or worms in the body in association with the lesion, or (b) the presence of a dead or disintegrated worm, or (c) a lesion persisting after the complete absorption of a worm.
- (4) The *presence* of signs of filarial disease with microfilaria in the blood does not necessarily imply that the parent of the embryos is the cause of the lesion. The worm that caused the lesion may be dead or alive; if the latter, it may not have embryos in the blood.
- (5) The *absence* of signs of filarial disease without microfilaria in the blood does not necessarily imply an absence of adult worms, as the worms may not have been present in the body sufficiently long to produce (*ex hypothesi*) a lesion.

TABLE I.

Shewing percentage infected with microfilaria among those with and without signs of filarial disease* in Fij.

| | Number examined | Number infected with microfilaria | Percentage |
|--|--------------------|---|------------|
| With signs of filarial disease | 217 | 152 | 36.4 |
| Without signs of filarial disease | 527 | 103 | 19.5 |

* The only 'signs of disease' considered throughout this paper are enlarged lymphatic glands, hydrocele, enlarged testis, abscess, and elephantiasis.

Conclusion: Microfilaria is commoner among those with signs of filarial disease than among those without signs of filarial disease.

For a further analysis of this table, *vide* Appendix, Table XII.

TABLE II.

Shewing percentage infected with microfilaria among those with and without particular signs of filarial disease in Fiji.

| | Number examined | Number infected with microfilaria | Percentage |
|--------------------------------|--------------------|---|------------|
| With elephantiasis | 33 | 13 | 39'4 |
| Without elephantiasis | 911 | 247 | 27'1 |
| With enlarged glands | 325 | 118 | 36'3 |
| Without enlarged glands | 619 | 142 | 22'9 |
| With hydrocele | 50* | 20 | 40'0 |
| Without hydrocele | 462* | 142 | 30'7 |
| With enlarged testis | 34* | 11 | 32'3 |
| Without enlarged testis | 478* | 151 | 31'6 |
| With abscess† | 209 | 97 | 46'4 |
| Without abscess | 735 | 163 | 22'2 |

* Males.

† We understand from Dr. Manson-Bahr that in many cases cicatrices were taken as evidence of a pre-existing abscess.

Conclusion: Microfilaria is commoner among those with elephantiasis, enlarged glands, hydrocele, enlarged testis, or abscess than among those without these signs of filarial disease.

For a further analysis of the table, *vide* Appendix, Table XIII.

TABLE III.

Shewing percentage exhibiting signs of filarial disease among those with and without microfilaria, in Fiji.

| | Number examined | Number with signs of filarial disease | Percentage |
|-----------------------------|--------------------|---|------------|
| With microfilaria | 260 | 152 | 58'4 |
| Without microfilaria | 684 | 269 | 39'3 |

Conclusion: Signs of filarial disease are commoner among those infected with microfilaria than among those not infected with microfilaria.

For a further analysis of this table, *vide* Appendix, Table XIV.

TABLE IV.

Shewing percentage exhibiting particular signs of filarial disease among those with and without microfilaria, in Fiji.

| | Number examined | Number with particular signs of filarial disease | Percentage |
|-----------------------------|--------------------|--|------------|
| | | ELEPHANTIASIS | |
| With microfilaria | 260 | 13 | 5.0 |
| Without microfilaria | 684 | 20 | 2.9 |
| | | GLANDS | |
| With microfilaria | 260 | 118 | 45.3 |
| Without microfilaria | 684 | 207 | 30.2 |
| | | HYDROCELE * | |
| With microfilaria | 162* | 20 | 12.3 |
| Without microfilaria | 350* | 30 | 8.5 |
| | | ENLARGED TESTIS | |
| With microfilaria | 162* | 11 | 6.7 |
| Without microfilaria | 350* | 21 | 6.0 |
| | | ABSCESS | |
| With microfilaria | 260 | 97 | 37.3 |
| Without microfilaria | 684 | 112 | 16.3 |

* Males.

Conclusion: Cases of elephantiasis, enlarged glands, hydrocele, abscess, and enlarged testis, respectively, are commoner among those infected with microfilaria than among those not infected with microfilaria.

For a further analysis of this table, *vide* Appendix, Table XV.

TABLE V.

Shewing percentage infected with microfilaria and percentage shewing signs of disease at various age periods in the population examined, in Fiji

| Age period | Number examined | Percentage infected with microfilaria | Percentage shewing signs of filarial disease | |
|------------|-----------------|---------------------------------------|--|-------|
| MALE | | | | |
| 1-10 | 85 | 1'2 | 18'8 | 18'8* |
| 11-20 | 117 | 21'4 | 59'0 | 59'0* |
| 21-30 | 108 | 39'8 | 64'8 | 64'8* |
| 31-40 | 83 | 47'0 | 78'3 | 78'3* |
| 41-50 | 63 | 52'4 | 69'8 | 66'6* |
| 51-60 | 35 | 37'1 | 71'4 | 62'8* |
| 61- | 21 | 38'1 | 76'2 | 61'9* |
| FEMALE | | | | |
| 1-10 | 66 | 10'6 | 9'1 | |
| 11-20 | 108 | 24'0 | 20'3 | |
| 21-30 | 124 | 22'6 | 31'4 | |
| 31-40 | 61 | 22'9 | 34'4 | |
| 41-50 | 41 | 34'1 | 26'8 | |
| 51-60 | 19 | 26'3 | 52'6 | |
| 61- | 13 | 30'7 | 23'0 | |

* Hydrocele and enlarged testis excluded.

Conclusion. No close relationship between the two sets of percentages is evident.

Manson-Bahr investigated five areas in Fiji. It is interesting to compare the microfilaria rate with the filarial disease rate in the areas.

TABLE VI.

Shewing percentage infected with microfilaria and percentage shewing signs of disease in various Fijian localities.

| Locality | Number examined | Percentage infected with microfilaria | Percentage with signs of filarial disease (including elephantiasis) | Percentage with elephantiasis |
|-------------------------|-----------------|---------------------------------------|---|-------------------------------|
| Bau | 169 | 13·0 | 28·9 | 0·0 |
| Oneata | 114 | 24·6 | 38·6 | 1·8 |
| Lakemba Villages | 178 | 24·7 | 48·8 | 5·0 |
| Lakemba Town | 264 | 33·7 | 60·0 | 7·5 |
| Loma Loma | 219 | 35·6 | 35·1 | 1·8 |

Conclusion: No close relationship between the two sets of percentages is evident.

Dr. Manson-Bahr has also kindly furnished me with unpublished observations made by him in Ceylon. From these I have compiled the following tables.

TABLE VII.

Shewing percentage infected with microfilaria among those with and without signs of filarial disease, in Ceylon.

| | Number examined | Number infected with microfilaria | Percentage |
|--|-----------------|-----------------------------------|------------|
| With signs of filarial disease | 52 | 4 | 7·7 |
| Without signs of filarial disease | 1256 | 39 | 3·1 |

TABLE VIII.

Shewing percentage infected with microfilaria among those with and without particular signs of filarial disease, in Ceylon. *

| | Number examined | Number infected with microfilaria | Percentage |
|--------------------------------|--------------------|---|------------|
| With elephantiasis | 46 | 2 | 4.3 |
| Without elephantiasis | 1262 | 41 | 3.2 |
| With enlarged glands | 35 | 2 | 5.7 |
| Without enlarged glands | 1273 | 41 | 3.2 |
| With hydrocele | 7* | 0 | 0 |
| Without hydrocele | 957* | 40 | 4.2 |
| With abscess | 1 | 0 | 0 |
| Without abscess | 1307 | 43 | 3.3 |

* Males.

NOTE :—No record is made of enlarged testis.

Conclusion: Microfilaria is commoner among those with elephantiasis, enlarged glands, or hydrocele than among those without these signs of filarial disease.

TABLE IX.

Shewing percentage exhibiting signs of filarial disease among those with and without microfilaria, in Ceylon.

| | Number examined | Number with signs of filarial disease | Percentage |
|-----------------------------|--------------------|---|------------|
| With microfilaria | 43 | 4 | 9.3 |
| Without microfilaria | 1265 | 48 | 3.8 |

Conclusion: Signs of filarial disease are commoner among those infected with microfilaria than among those not infected with microfilaria.

TABLE X.

Shewing percentage exhibiting particular signs of filarial disease among those with and without microfilaria, in Ceylon.

| | Number examined | Number with particular signs of filarial disease | Percentage |
|-----------------------------|-----------------|--|------------|
| With microfilaria | 43 | ELEPHANTIASIS 2 | 4·6 |
| Without microfilaria | 1265 | 44 | 3·5 |
| With microfilaria | 43 | ENLARGED GLANDS 2 | 4·6 |
| Without microfilaria | 1265 | 33 | 2·6 |
| With microfilaria | 40* | HYDROCELE 0 | 0 |
| Without microfilaria | 924* | 7 | 0·7 |
| With microfilaria | 43 | ABSCESS 0 | 0 |
| Without microfilaria | 1265 | 1 | 0·08 |

* Males. NOTE.—No record is made of enlarged testis.

Conclusion: Elephantiasis and enlarged glands are commoner among those infected with microfilaria than among those not infected with microfilaria; the reverse holds true for hydrocele.

Finally a comparison may be made between the various rates obtained for Fiji and Ceylon.

TABLE XI.

Shewing various rates in Fiji and Ceylon.

| | FIJI | CEYLON |
|-----------------------------|--------------------|-------------------|
| Number examined | 944 | 1308 |
| Microfilaria rate | Percentage 27·0 | Percentage 3·3 |
| Enlarged glands rate | 34·4 | 2·6 |
| Hydrocele rate | 9·7* | 0·7* |
| Abscess rate | 22·1 | 0·08 |
| Enlarged testis rate | 6·2* | 0·0* |
| Elephantiasis rate | 3·4 | 3·5 |

* Male rate

Conclusion The microfilaria rate of those examined in Fiji is higher than that of those examined in Ceylon, and the rates for enlarged glands, hydrocele and abscess are also higher, but there is no evident correlation between the two sets of figures. Although the microfilaria rate of those examined in Fiji is more than eight times as great as that of those examined in Ceylon, yet the elephantiasis rates are the same.

While it is permissible—given the microfilaria rate of those examined in each of two countries—to compare other rates solely in relation to the respective microfilaria rates, it is not permissible to compare independently any two corresponding rates, *e g*, the microfilaria rates, unless we know that they are derived from homogeneous populations. Thus, there appears to be evidence that the microfilaria rates, in some countries at least, vary with the age periods, so that in such cases the rates could not be compared unless we knew that each population examined contained, *e g*, the same proportion of children under ten, but if we restrict our comparison to people of a particular age period (and sex), then the comparison is, within limits, valid. Further, it is not permissible, in strictness, to speak of the microfilaria rate of a country, unless we examine the whole population, as was virtually done by Manson-Bahr in certain of the Fijian islands. Where this is impossible, if we wish to get a figure representing the microfilaria rate of a country, we must select our population in proportion to the *number of people alive at each age period*. This latter information can only be obtained from the census figures which, even where they exist in the tropics, are of doubtful accuracy.

SUMMARY

1. Microfilaria is commoner among those with signs of filarial disease than among those without signs of filarial disease.
2. Signs of filarial disease are commoner among those infected with microfilaria than among those not infected with microfilaria.
3. There is no evident correlation between various microfilaria rates and the corresponding filarial disease rates.

REFERENCE

- BAHR, P H (1912) Filariasis and Elephantiasis in Fiji, being a report to the London School of Tropical Medicine London Witherby & Co

TABLE XII.
(Analysis of Table I).

Shewing percentage infected with microfilaria among those with and without signs of filarial disease, at different age periods, in Fiji.

| Age period | | MALE | | | FEMALE | | |
|------------|-------------------|-----------------|-----------------------------------|---------------------------------------|-----------------|-----------------------------------|---------------------------------------|
| | | Number examined | Number infected with microfilaria | Percentage infected with microfilaria | Number examined | Number infected with microfilaria | Percentage infected with microfilaria |
| 1-10 | With signs ... | 16 | 0 | 0'0 | 6 | 2 | 33'3 |
| | Without signs ... | 69 | 1 | 1'4 | 60 | 5 | 8'3 |
| 11-20 | With signs ... | 69 | 17 | 24'6 | 22 | 7 | 31'8 |
| | Without signs ... | 48 | 8 | 16'6 | 86 | 19 | 22'1 |
| 21-30 | With signs ... | 70 | 30 | 42'8 | 39 | 9 | 23'0 |
| | Without signs ... | 38 | 13 | 34'2 | 85 | 19 | 22'3 |
| 31-40 | With signs ... | 65 | 34 | 52'3 | 21 | 5 | 23'8 |
| | Without signs ... | 18 | 5 | 28'0 | 40 | 9 | 22'5 |
| 41-50 | With signs ... | 44 | 24 | 54'5 | 11 | 5 | 45'4 |
| | Without signs .. | 19 | 9 | 47'4 | 30 | 9 | 30'0 |
| 51-60 | With signs ... | 25 | 10 | 40'0 | 10 | 2 | 20'0 |
| | Without signs .. | 10 | 3 | 30'0 | 9 | 3 | 33'3 |
| 61- | With signs ... | 16 | 6 | 37'5 | 3 | 1 | 33'3 |
| | Without signs .. | 5 | 2 | 40'0 | 10 | 3 | 30'0 |

TABLE XIII.
(Analysis of Table II).

Showing the percentage infected with microfilaria among those with and without particular signs of filarial disease at different age periods, in Fiji.

| Age period | | MALE | | | FEMALE | | |
|------------|---------------------------|-----------------|-----------------------------------|---------------------------------------|-----------------|-----------------------------------|---------------------------------------|
| | | Number examined | Number infected with microfilaria | Percentage infected with microfilaria | Number examined | Number infected with microfilaria | Percentage infected with microfilaria |
| 1-10 | With abscess ... | 2 | 0 | 0.0 | 0 | 0 | 0.0 |
| | Without abscess ... | 83 | 1 | 1.2 | 66 | 7 | 1.1 |
| | With glands ... | 15 | 0 | 0.0 | 6 | 2 | 33.3 |
| | Without glands ... | 70 | 1 | 1.4 | 60 | 5 | 8.3 |
| | With hydrocele ... | 0 | 0 | 0.0 | ... | ... | ... |
| | Without hydrocele ... | 85 | 1 | 1.2 | ... | ... | ... |
| | With testis ... | 0 | 0 | 0.0 | ... | ... | ... |
| | Without testis ... | 85 | 1 | 1.2 | ... | ... | ... |
| 11-20 | With elephantiasis ... | 0 | 0 | 0.0 | 0 | 0 | 0.0 |
| | Without elephantiasis ... | 85 | 1 | 1.2 | 66 | 7 | 1.1 |
| | With abscess ... | 21 | 7 | 33.3 | 10 | 3 | 30.0 |
| | Without abscess ... | 96 | 18 | 18.7 | 98 | 23 | 23.5 |
| | With glands ... | 61 | 14 | 22.9 | 14 | 4 | 28.6 |
| | Without glands ... | 56 | 11 | 19.6 | 94 | 22 | 23.4 |
| | With hydrocele ... | 0 | 0 | 0.0 | ... | ... | ... |
| | Without hydrocele ... | 117 | 25 | 21.3 | ... | ... | ... |
| 21-30 | With testis ... | 1 | 0 | 0.0 | ... | ... | ... |
| | Without testis ... | 116 | 25 | 21.5 | ... | ... | ... |
| | With elephantiasis ... | 0 | 0 | 0.0 | 0 | 0 | 0.0 |
| | Without elephantiasis ... | 117 | 25 | 21.3 | 108 | 26 | 24.0 |
| | With abscess ... | 39 | 18 | 46.1 | 16 | 4 | 25.0 |
| | Without abscess ... | 69 | 25 | 36.2 | 108 | 24 | 22.2 |
| | With glands ... | 57 | 25 | 43.8 | 26 | 6 | 23.0 |
| | Without glands ... | 51 | 18 | 35.3 | 98 | 22 | 22.4 |
| 31-40 | With hydrocele ... | 6 | 0 | 0.0 | ... | ... | ... |
| | Without hydrocele ... | 102 | 43 | 42.1 | ... | ... | ... |
| | With testis ... | 4 | 1 | 25.0 | ... | ... | ... |
| | Without testis ... | 104 | 42 | 40.4 | ... | ... | ... |
| | With elephantiasis ... | 3 | 1 | 33.3 | 0 | 0 | 0.0 |
| | Without elephantiasis ... | 105 | 42 | 40.0 | 124 | 28 | 22.6 |

TABLE XIII—continued

| Age period | | MALE | | | FEMALE | | |
|------------|---------------------------|-----------------|-----------------------------------|---------------------------------------|-----------------|-----------------------------------|---------------------------------------|
| | | Number examined | Number infected with microfilaria | Percentage infected with microfilaria | Number examined | Number infected with microfilaria | Percentage infected with microfilaria |
| 31-40 | With abscess ... | 43 | 28 | 65.1 | 11 | 3 | 27.2 |
| | Without abscess ... | 40 | 11 | 27.5 | 50 | 11 | 22.0 |
| | With glands ... | 54 | 27 | 50.0 | 12 | 3 | 25.0 |
| | Without glands ... | 29 | 12 | 41.4 | 49 | 11 | 22.4 |
| | With hydrocele ... | 18 | 7 | 38.9 | ... | ... | ... |
| | Without hydrocele ... | 65 | 32 | 49.2 | ... | ... | ... |
| | With testis ... | 4 | 3 | 75.0 | ... | ... | ... |
| | Without testis ... | 79 | 36 | 45.5 | ... | ... | ... |
| | With elephantiasis ... | 7 | 4 | 57.1 | 3 | 0 | 0.0 |
| | Without elephantiasis ... | 76 | 35 | 46.0 | 58 | 14 | 24.1 |
| 41-50 | With abscess ... | 31 | 17 | 54.8 | 8 | 5 | 62.5 |
| | Without abscess ... | 32 | 16 | 50.0 | 33 | 9 | 27.3 |
| | With glands ... | 37 | 20 | 54.0 | 7 | 3 | 42.8 |
| | Without glands ... | 26 | 13 | 50.0 | 34 | 11 | 32.3 |
| | With hydrocele ... | 15 | 10 | 66.6 | ... | ... | ... |
| | Without hydrocele ... | 48 | 23 | 47.9 | ... | ... | ... |
| | With testis ... | 10 | 2 | 20.0 | ... | ... | ... |
| | Without testis ... | 53 | 31 | 58.5 | ... | ... | ... |
| | With elephantiasis ... | 6 | 2 | 33.3 | 1 | 1 | 100.0 |
| | Without elephantiasis ... | 57 | 31 | 54.4 | 40 | 13 | 32.5 |
| 51-60 | With abscess ... | 14 | 9 | 64.3 | 5 | 0 | 0.0 |
| | Without abscess ... | 21 | 4 | 19.0 | 14 | 5 | 35.7 |
| | With glands ... | 19 | 9 | 47.4 | 6 | 1 | 16.6 |
| | Without glands ... | 16 | 4 | 25.0 | 13 | 4 | 30.7 |
| | With hydrocele ... | 10 | 4 | 40.0 | ... | ... | ... |
| | Without hydrocele ... | 25 | 9 | 36.0 | ... | ... | ... |
| | With testis ... | 7 | 2 | 28.5 | ... | ... | ... |
| | Without testis ... | 28 | 11 | 39.3 | ... | ... | ... |
| | With elephantiasis ... | 7 | 3 | 42.8 | 2 | 1 | 50.0 |
| | Without elephantiasis ... | 28 | 10 | 35.7 | 17 | 4 | 23.5 |
| 61- | With abscess ... | 7 | 2 | 28.5 | 3 | 1 | 33.3 |
| | Without abscess ... | 14 | 6 | 42.8 | 10 | 3 | 30.0 |
| | With glands ... | 11 | 4 | 36.3 | 0 | 0 | 0.0 |
| | Without glands ... | 10 | 4 | 40.0 | 13 | 4 | 30.7 |
| | With hydrocele ... | 2 | 0 | 0.0 | ... | ... | ... |
| | Without hydrocele ... | 19 | 8 | 42.1 | ... | ... | ... |
| | With testis ... | 8 | 3 | 37.5 | ... | ... | ... |
| | Without testis ... | 13 | 5 | 38.4 | ... | ... | ... |
| | With elephantiasis ... | 4 | 1 | 25.0 | 1 | 0 | 0.0 |
| | Without elephantiasis ... | 37 | 7 | 18.9 | 12 | 4 | 33.3 |

TABLE XIV.
(Analysis of Table III).

Shewing percentage exhibiting signs among those with and without microfilaria, at different age periods, in Fiji.

| Age period | | MALE | | | FEMALE | | |
|------------|-----------------------|-----------------|------------|------------|-----------------|------------|------------|
| | | Number examined | With signs | Percentage | Number examined | With signs | Percentage |
| 1-10 | With microfilaria .. | 1 | 0 | 0'0 | 7 | 2 | 28'6 |
| | Without microfilaria | 84 | 16 | 19'0 | 59 | 4 | 6'8 |
| 11-20 | With microfilaria .. | 25 | 17 | 68'0 | 26 | 7 | 27'0 |
| | Without microfilaria | 92 | 52 | 56'5 | 82 | 15 | 18'3 |
| 21-30 | With microfilaria .. | 43 | 30 | 69'7 | 28 | 9 | 32'1 |
| | Without microfilaria | 65 | 40 | 61'5 | 96 | 30 | 31'2 |
| 31-40 | With microfilaria .. | 39 | 34 | 87'2 | 14 | 5 | 35'7 |
| | Without microfilaria | 44 | 31 | 70'5 | 47 | 16 | 34'0 |
| 41-50 | With microfilaria .. | 33 | 24 | 72'1 | 14 | 5 | 35'7 |
| | Without microfilaria | 30 | 20 | 66'6 | 27 | 6 | 22'2 |
| 51-60 | With microfilaria .. | 13 | 10 | 76'9 | 5 | 2 | 40'0 |
| | Without microfilaria | 22 | 15 | 68'1 | 14 | 8 | 57'1 |
| 61- | With microfilaria ... | 8 | 6 | 75'0 | 4 | 1 | 25'0 |
| | Without microfilaria | 13 | 10 | 76'9 | 9 | 2 | 22'2 |

TABLE XV.

(Analysis of Table IV).

Showing percentage exhibiting particular signs of filarial disease among those with and without filarial disease, at different age periods in Fiji.

| Age period | | MALE | | | | | | FEMALE | | | |
|------------|--------------------------|-----------------|---------|--------|-----------|--------|---------------|-----------------|---------|--------|---------------|
| | | Number examined | Abscess | Glands | Hydrocele | Testis | Elephantiasis | Number examined | Abscess | Glands | Elephantiasis |
| 1-10 | With microfilaria ... | 1 | 0 | 0 | 0 | 0 | 0 | 7 | 0 | 28.6 | 0 |
| | Without microfilaria ... | 84 | 2.4 | 17.8 | 0 | 0 | 0 | 59 | 0 | 6.8 | 0 |
| 11-20 | With microfilaria ... | 25 | 28.0 | 56.0 | 0 | 0 | 0 | 26 | 11.5 | 15.4 | 0 |
| | Without microfilaria ... | 92 | 15.2 | 51.1 | 0 | 1.1 | 0 | 82 | 8.5 | 12.2 | 0 |
| 21-30 | With microfilaria ... | 43 | 41.8 | 58.1 | 0 | 2.3 | 2.3 | 28 | 14.3 | 21.4 | 0 |
| | Without microfilaria ... | 65 | 32.3 | 49.2 | 9.2 | 1.5 | 1.5 | 96 | 11.4 | 20.8 | 0 |
| 31-40 | With microfilaria ... | 39 | 71.8 | 69.2 | 15.4 | 7.7 | 10.2 | 14 | 21.4 | 21.4 | 0 |
| | Without microfilaria ... | 44 | 34.1 | 61.4 | 25.0 | 2.3 | 6.8 | 47 | 17.0 | 19.1 | 6.4 |
| 41-50 | With microfilaria ... | 33 | 51.5 | 60.6 | 30.3 | 6.1 | 6.1 | 14 | 35.7 | 21.4 | 7.1 |
| | Without microfilaria ... | 30 | 46.6 | 56.6 | 16.6 | 26.6 | 13.3 | 27 | 11.1 | 14.8 | 0 |
| 51-60 | With microfilaria ... | 13 | 69.2 | 69.2 | 30.7 | 15.4 | 23.1 | 5 | 0 | 20.0 | 20.0 |
| | Without microfilaria ... | 22 | 22.7 | 45.4 | 27.3 | 22.7 | 18.2 | 14 | 35.7 | 35.7 | 7.1 |
| 61- | With microfilaria ... | 8 | 25.0 | 50.0 | 0 | 37.5 | 12.5 | 4 | 25.0 | 0 | 0 |
| | Without microfilaria ... | 13 | 38.4 | 53.8 | 15.4 | 38.5 | 23.1 | 9 | 22.2 | 0 | 11.1 |

A NEW SPECIES OF CESTODE (*ANOPLOCEPHALA VULGARIS*) FROM AN AFRICAN RHINOCEROS

BY

T. SOUTHWELL

(Received for publication 8 December, 1920)

Twelve specimens, a large number of fragments, and several single segments, were obtained by Professor Yorke on 23rd August, 1912, from a rhinoceros (*Rhinoceros bicornis*), at Ngoa, N.E. Rhodesia.

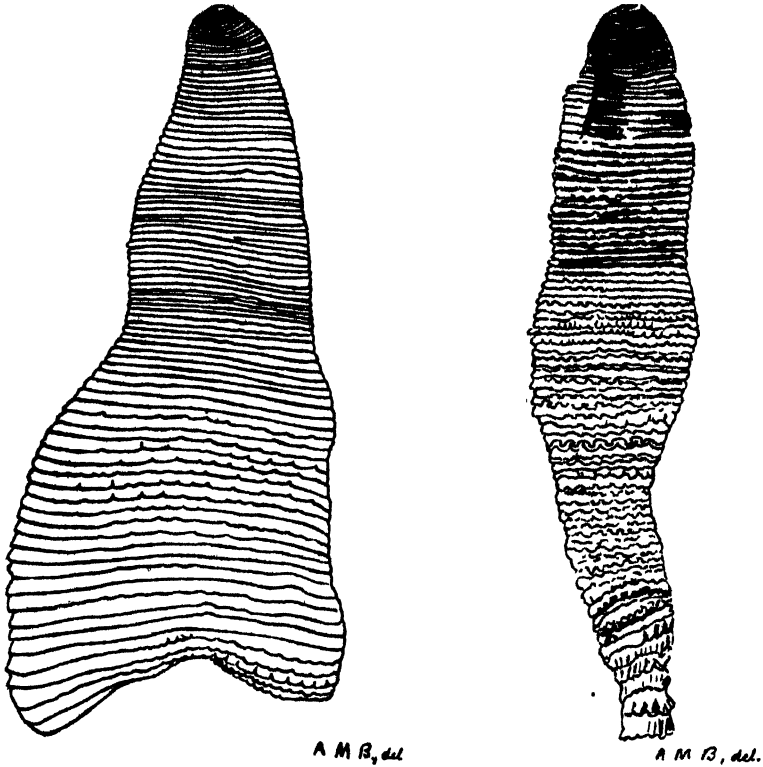
EXTERNAL CHARACTERS. Probably this worm does not exceed a length of about 12 cm. The measurement of six of the largest specimens were as follows:—

| Length | Greatest breadth | Greatest thickness | Head length | Head breadth | No. of Segments |
|--------|------------------|--------------------|-------------|--------------|-----------------|
| 75 mm. | 27 mm. | 1.0 mm. | 0.75 mm. | 1.0 mm. | 150 |
| 67 mm. | 16 mm. | 1.25 mm. | 0.5 mm. | 1.0 mm. | 143 |
| 60 mm. | 15 mm. | 0.75 mm. | 1.1 mm. | 1.25 mm. | 117 |
| 48 mm. | 11 mm. | 1.5 mm. | 0.5 mm. | 1.1 mm. | 114 |
| 47 mm. | 16 mm. | 0.75 mm. | no head | | 125 |
| 44 mm. | 17 mm. | 0.75 mm. | 0.5 mm. | 1.0 mm. | 121 |

The worms vary in shape, as will be seen from figs. 1 and 2. In some, the posterior segments are very broad (27 mm.), and short (1 mm.), whilst in others they are narrow (4 mm.) and long (1.25 mm). The segments overlap each other, and the free edges are frilled, the frilling becoming much more pronounced posteriorly. The genital pores are all dextral.

HEAD. The head is very small (figs. 1, 2 and 3). The four suckers are directed forward and slightly outward. Their diameter is about 390μ , and the muscular rim has a thickness of about 80μ . There are no lappets, and there is no neck. The lateral margins of

the anterior segments curve forward so that the head rests in a deep depression between two shoulders, and can be seen only with difficulty with the naked eye (figs. 1 and 2).



FIGS. 1 and 2. Two specimens shewing variation in shape. ($\times 2$)

MUSCULAR SYSTEM. The muscular system is poorly developed; the longitudinal bundles have a thickness of about 50μ , and the annular bundles of 15μ ; a single bundle of muscle fibres connects the

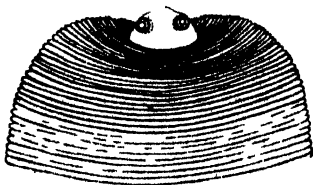


FIG. 3. Head and anterior segments. ($\times 10$)

internal extremity of the cirrus bulb to the ventral wall. The dorso-ventral muscle fibres are strongly developed, and extend, at irregular intervals, from the dorsal to the ventral surface (figs. 5 and 8).

NERVOUS SYSTEM. There are three longitudinal nerves on each side, the main nerve being median. The other two are small and are situated lateral to the main nerve, one dorsal and one ventral (figs. 4 and 5).

WATER VASCULAR SYSTEM. Only a single vessel could be made out with certainty on each side. It was well developed, and had a diameter of about 45μ . Numerous branches were to be seen, especially laterally (figs. 4 and 5).

MALE GENITALIA. *Testes* (figs. 4, 5 and 6). These first appear in segment 15, and they have disappeared in segment 62. At first they are small, each testis having a diameter of about 20μ only.

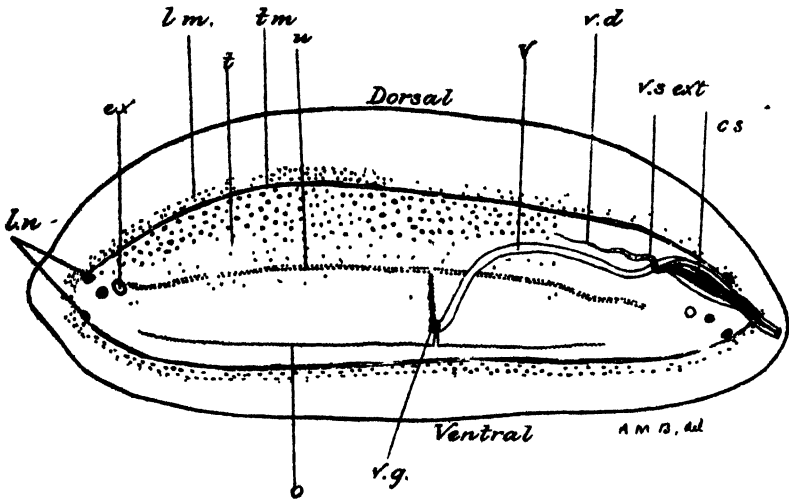


FIG 4. Immature segment shewing developing of genitalia. *c.s.*—cirrus sac; *ex.*—ventral excretory vessel; *l.m.*—longitudinal muscles; *l.n.*—lateral nerves; *o.*—ovary; *t.*—testes; *t.m.*—transverse muscles; *u.*—uterus; *v.*—vagina; *v.d.*—vas deferens; *v.g.*—vitelline glands; *v.s. ext.*—external vesicula seminalis. ($\times 28$.)

They attain their maximum development between segments 18 and 24, where each testis measures about 62μ by 30μ . When fully developed, they extend the whole distance between the aporal water vessel and the inner extremity of the cirrus bulb. While the bulk of the testes lies dorsally, a number of acini extend quite ventrally and reach the rudiment of the ovaries.

Vas deferens. The cirrus bulb is first evident in segment 10, where it measures about 250μ by 150μ . The rudiment of the outer seminal vesicle is also to be seen in this segment, lying immediately

internal to the cirrus bulb (fig. 4). In segment 23, the cirrus bulb has enlarged to 900μ , and its breadth is 275μ ; it lies dorsal to the water vessel and nerve and gradually curves ventrally, until its internal extremity lies almost on the ventral surface. The outer seminal vesicle lies internal and dorsal to the cirrus bulb; it is a U-shaped tube having a diameter of about 40μ , the limbs of which lie close together. The inner limb gradually merges into the vas deferens, which narrows and pursues a wavy course along the dorsal surface. The inner seminal vesicle is first visible in segment 26, as a small club-shaped cavity near the internal extremity of the cirrus bulb; it enlarges rapidly, like the cirrus bulb itself, and

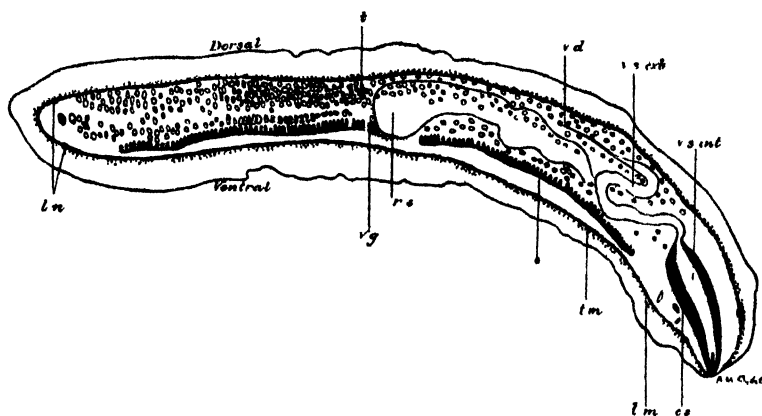


FIG. 5. Mature segment shewing genitalia. *c.s.*—cirrus sac; *l.m.*—longitudinal muscles; *l.n.*—lateral nerves; *o.*—ovary; *r.s.*—receptaculum seminis; *t.*—testes; *t.m.*—transverse muscles; *v.d.*—vas deferens; *v.g.*—vitelline glands; *v.s. ext.*—external vesicula seminalis; *v.s. int.*—internal vesicula seminalis. ($\times 14$.)

in segment 37 practically fills the entire cirrus bulb. The cirrus shortens as the inner seminal vesicle enlarges, and eventually disappears altogether. No spines were seen on the cirrus.

In segment 50 the cirrus bulb is about 2 mm. long and has a diameter of 0.45 mm. It continues of this size up to about segment 80, when it gradually becomes straighter and narrower; it persists to the last segment. The outer seminal vesicle also enlarges enormously and alters its position accordingly up to segment 46, after which it gradually shrinks.

FEMALE GENITALIA. *Ovary* (figs. 4, 5 and 6). This first appears in segment 19; it is situated ventrally and measures 45μ

in the dorso-ventral diameter. It attains its highest development between segments 37 and 50, and disappears in segment 64. When fully developed, it extends laterally to within 650μ of the aporal water vessel, and to within 700μ of the poral water vessel. The ovary is divided into two wings by the vitelline glands; the poral wing has a lateral diameter of about 2.2 mm. , and the aporal wing of 3.5 mm. (figs. 5 and 6). The median axis of the ovary is very slightly on the pore side of the segment.

The ovary consists of a series of club-shaped acini arising from a ventral horizontal base (fig. 6); the larger acini measure about

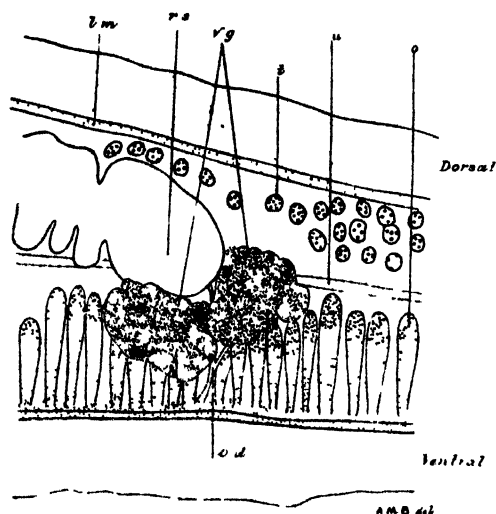


FIG. 6. Centre of a mature segment. *l.m.*—longitudinal muscles; *o.*—ovary; *o.d.*—oviduct; *r.s.*—receptaculum seminis; *t.*—testes; *u.*—uterus; *v.g.*—vitelline glands. ($\times 35$.)

470μ dorso-ventrally, and 60μ laterally. They decrease in size towards the periphery of the ovary to a slight extent only.

Receptaculum and Vagina. In segment 12 the vagina is well defined as a clear irregular tube having a diameter of about 70μ , and in segment 14 the receptaculum is seen as a slight dilatation of the median extremity of the vagina. Both the vagina and the receptaculum increase in size rapidly, and become enormously distended; in segment 29 the vagina has a diameter of about 450μ , and the receptaculum fills the whole dorso-ventral area (fig. 5). After segment 51, both these structures atrophy quickly. The

vagina has the following relationship to the cirrus bulb; from the genital pore it runs inwards, ventral to the bulb, but dorsal to the excretory vessel and nerve: it then crosses posterior to the cirrus bulb and runs dorsal to it.

In the median direction the receptaculum is continued as a narrow tube, which is joined by the oviduct and continues in a dorsal direction as a long fertilisation canal to the uterus. After the vitelline glands and receptaculum seminis are well developed, they hide the other structures in the vicinity, but it was noted that the vitelline duct opens near the junction of the oviduct and fertilisation canal, posterior and ventral to the receptaculum seminis. The relative position of these ducts is shewn diagrammatically in fig. 7.

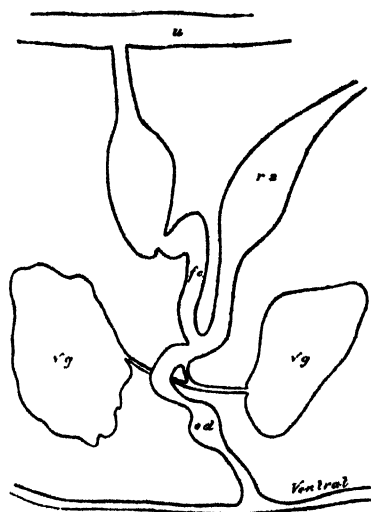


FIG. 7. Diagrammatic representation of the connections of the receptaculum seminis, ovary, oviduct, vitelline glands and uterus. *f.c.*—fertilisation canal; *o.d.*—oviduct; *r.s.*—receptaculum seminis; *u.*—uterus; *v.g.*—vitelline glands.

Vitelline glands. The rudiment of the vitelline glands appears with that of the receptaculum in segment 8, and in segment 14 a few small vitelline acini are present. The vitelline glands practically disappear in segment 94, but traces of them persist up to segment 99. They reach their maximum development between segments 44 and 50, and consist of two definite wings, separated from each other and presenting a V-shaped appearance. The poral wing is smaller than the aporal wing, the former measuring about 370μ by 200μ ,

and the latter 390μ by 390μ ; each is lobulated. Both wings lie on, but not touching, the ventral surface (figs. 6 and 7).

Shell glands. The shell gland consists in segments 24 to 27 of a thickening on the wall of the fertilisation canal, which measures about 75μ by 55μ . In posterior segments it could not be found.

Uterus. The rudiment of the uterus is to be seen in segment 7 or 8. In segment 17, it consists of a very faint cell-string running midway between the dorsal and ventral surfaces (fig. 4). Its future development was followed with some difficulty, owing to the fact that the testes, ovaries and receptaculum masked its presence. In segment 28, it runs between the ovaries and the testes as a straight tube from one water vessel to the other. In segment 48, it has enlarged a little and its course has become undulating. In succeeding segments, the undulations become more pronounced, and in about segment 70 it presents the appearance of a number of vertical tubes, not always clearly separated from each other ventrally and dorsally, and containing immature eggs (fig. 8). Laterally, the extremities

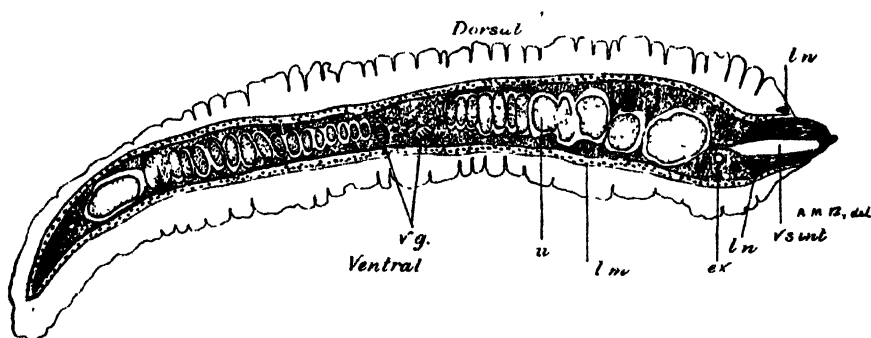


FIG. 8. Gravid uterus, posterior view, shewing isolated compartments into which the uterus is divided. *ex.*—ventral excretory vessel; *l.m.*—longitudinal muscles; *l.n.*—lateral nerves; *u.*—uterus; *v.g.*—vitelline glands; *v.s. int.*—internal vesicula seminalis. ($\times 14$.)

of the uterus remain straight and dilated. In the posterior segments the uterus fills the proglottid entirely, and dorso-ventral and antero-posterior muscular partitions can be seen with great clearness in whole segments or in sections viewed either anteriorly, posteriorly, dorsally or ventrally. No sterile segments were observed.

Eggs. The eggs enlarge and mature gradually in the posterior segments, the pyriform apparatus appearing last. The mature eggs

in preserved specimens are of different shapes and sizes, a condition which appears to be dependent on reciprocal pressure in the uterus. Extreme types are either ovoid or cuboid, the latter predominating (fig. 9), but intermediate types occur in abundance. In preserved specimens the egg has the following dimensions:—Size of egg, 77μ to 95μ . Thickness of outer envelope, 16μ to 18μ . Diameter of embryo, 18μ to 19μ . Length of horns of pyriform apparatus, 18μ . The free egg in the fresh condition is undoubtedly spherical.

In immature eggs the middle envelope lies close to the outer envelope. As the egg matures the middle envelope gradually shrinks until it becomes a small mass, about 1μ to 2μ in diameter, attached to the filaments of the pyriform apparatus. Its size,

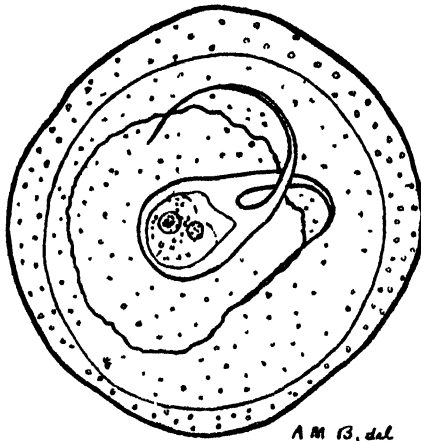


FIG. 9. Eggs from gravid uterus. ($\times 600$)

therefore, cannot be given. The eggs contain numerous yolk particles and granular material. The horns cross each other in very mature eggs, and each horn grows out into a long filament which becomes applied to the outer face of the vitelline envelope. The segments drop off either singly or in clusters of two, three, or four. When single they assume peculiar shapes.

DIAGNOSIS. Peters, in 1856, published a description of the external characters of a tapeworm from an African rhinoceros from Mozambique, to which he gave the name *T. gigantea*. The length of the worm was stated to be 12 cms. and the maximum breadth

as 27 to 29 mm. Some years later, Murie (1870) wrote 'A couple of years ago some dozen joints of what I may safely term an enormous tapeworm were placed in my hands by Mr. Bartlett, they having been passed by the young male *Rhinoceros indicus* in the Gardens.' He gives a brief description of the external characters of the segments, and named the worm *T. magna* (?). The following year (1871) Peters stated that Murie's *T. magna* was the same as his *T. gigantea*. The worm was transferred to genus *Anoplocephala* by Blanchard in 1891.

The next paper dealing with Cestodes from a Rhinoceros is that of the MacCallums (1912); they give a detailed account of the external and internal anatomy of the segments of an enormous worm at least 20 feet long passed by a Javanese rhinoceros (*R. sondaicus*). The MacCallums assume that their worm is the same as that described by Peters and Murie.

Douthitt, in his monograph of the *Anoplocephalidae* (1915) refers to the MacCallums' paper, and states that he considers the worm should be transferred to genus *Schizotaenia*.

Whether the MacCallums were correct in their inference that the worm found by them in *Rhinoceros sondaicus* is identical with those found by Peters and Murie in the African rhinoceros and Indian rhinoceros, respectively, seems to be a matter of some doubt, having consideration to the enormous difference in size, but, as neither Peters nor Murie give any detailed account of the internal anatomy of these worms, it is impossible to form any definite judgment.

It should be noted that the worm with which we are dealing conforms, as regards size, much more closely to Peters' worm than to the MacCallums'.

To avoid confusion, it appears to be best to associate the name *A. gigantea* with the worms described in detail by the MacCallums.

There is, however, no doubt that the worm with which we are dealing is different from that described by the MacCallums. The chief points of difference are shewn in the following table:—

| | <i>A. gigantea</i> | <i>A. vulgaris</i> , n. sp. |
|-----------------------------------|--|--|
| (1) Size | More than 20 feet | Probably not more than 12 cms. |
| (2) Gravid uterus | A large sacculated cavity which extends laterally almost from one margin of the segment to the other | Consists of a series of apparently more or less isolated compartments which originate from ingrowths of dorso-ventral and antero-posterior muscle fibres between the limbs of a convoluted tubular uterus. |
| (3) Cirrus | Armed | Unarmed |

I propose for this worm, which was obtained from *Rhinoceros bicornis*, the name *Anoplocephala vulgaris*, n.sp.

It should be noted that the MacCallums, both in their written description of their worm and in the illustration, have apparently confused the ovary and the vitelline glands and *vice versa*.

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STUDIES IN THE TREATMENT OF MALARIA

XXXI.—THE TIME OF ONSET OF THE PAROXYSMS IN SIMPLE TERTIAN MALARIA

BY

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The times recorded in the following paper are those at which a certain clinical event happened.

They are the hour,* when the patient's condition was such—shivering or feeling ill in one way or another—as to lead him to draw the Ward Sister's attention to the fact, or, on the other hand, the hour when the Sister herself noticed the patient's condition and proceeded to take his temperature, and to make a blood film.

Only those paroxysms are considered in which the temperature rose to over 100° F., and in which simple tertian parasites (*P. vivax*) were found in the blood films made at the time.

We are considering neither the exact time at which the patient's temperature began to rise nor that at which the maximum temperature was reached during the paroxysm, but simply, as already stated, the hour at which the patient's condition was such as to demand attention.

The following data in regard to the men's daily habits may be of importance in considering the causes which determine the onset of the paroxysm, although they cannot apply in certain cases to any

* e.g., 2 o'clock signifies 2.00—2.59. 3 o'clock, 3.00—3.59, etc.

but the first of a series of paroxysms, as after the first paroxysm the patient was sometimes not well enough to leave his bed and take ordinary diet before one or more subsequent paroxysms had occurred:—

| | | | | | |
|------------------|-----|-----|-----|-----|-------------|
| 7 a.m. | ... | ... | ... | ... | Reveille. |
| 8 a.m. | ... | ... | ... | ... | Breakfast. |
| 10 to 12-30 p.m. | ... | ... | ... | ... | Goes out. |
| 12-30 p.m. | ... | ... | ... | ... | Dinner. |
| 1 to 4-30 p.m. | ... | ... | ... | ... | Goes out. |
| 4-30 p.m. | ... | ... | ... | ... | Tea. |
| 9 p.m. | ... | ... | ... | ... | Lights out. |

In recording the hours at which the paroxysms were noted, it is possibly necessary to consider the fact that 'Summer time'* was in force during the years over which the observations extend, namely, 1917 when 'Summer time' began 2 a.m., 8th April, and ended 2 a.m., 17th September, and 1918 when it began 2 a.m., 24th March, and ended 2 a.m., 30th September.

As we did not know whether the change from 'Greenwich' to 'Summer' time, or rather the change in habits of life induced thereby, exerted any influence on the incidence of the paroxysms, we have grouped our observations into two periods, (1) those which occurred during the months October to March, when 'Greenwich' time was in operation, and (2) those which occurred during the months April to September, when 'Summer' time was in operation. Finally we have recorded the 'Greenwich' time of the paroxysms occurring during the months when 'Summer' time was in force.

Table I shews:—

(1) That for each winter month the maximum number of paroxysms occurred at 2 p.m. (Greenwich time), except in February, when thirteen paroxysms were recorded at 1 p.m. as against twelve at 2 p.m.

(2) That for each summer month the maximum number of paroxysms occurred at 2 p.m. ('Summer' time), and consequently,

(3) That for each summer month the maximum number of paroxysms occurred at 1 p.m. (Greenwich time).

The fact that the maximum number of paroxysms occurs at

* During 'Summer' time the clock was advanced one hour.

TABLE I.

Shewing the hours at which Simple Tertian Malaria Paroxysms occurred.

I. WINTER MONTHS: 'GREENWICH' TIME.

| Hours. | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | Noon | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | Mid- night |
|--------------|---|---|---|---|---|---|---|---|----|----|----|------|----|----|----|----|----|----|---|---|---|----|----|---------------|
| October ... | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 2 | 2 | 0 | 3 | 1 | 1 | 7 | 2 | 3 | 0 | 1 | 1 | 1 | 0 | 0 | 0 | 0 |
| November ... | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 2 | 1 | 3 | 0 | 0 | 0 | 4 | 0 | 0 | 2 | 1 | 0 | 0 | 0 | 2 | 0 | 0 |
| December ... | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 1 | 2 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| January ... | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 1 | 0 | 0 | 0 | 2 | 2 | 1 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 0 |
| February ... | 0 | 0 | 1 | 0 | 0 | 1 | 2 | 0 | 5 | 9 | 2 | 8 | 13 | 12 | 9 | 4 | 3 | 1 | 2 | 0 | 2 | 0 | 0 | 1 |
| March ... | 0 | 0 | 0 | 1 | 0 | 1 | 3 | 2 | 6 | 17 | 9 | 9 | 17 | 24 | 8 | 9 | 6 | 7 | 6 | 0 | 0 | 2 | 0 | 0 |
| | 0 | 1 | 1 | 1 | 0 | 3 | 6 | 6 | 15 | 31 | 14 | 18 | 32 | 51 | 21 | 17 | 11 | 11 | 9 | 1 | 2 | 4 | 0 | 1 |

II. SUMMER MONTHS: 'SUMMER' TIME.

| | | | | | | | | | | | | | | | | | | | | | | | | |
|---------------|---|---|---|---|---|---|----|----|----|----|----|----|----|-----|----|----|----|----|----|---|---|---|---|---|
| April ... | 0 | 0 | 1 | 0 | 0 | 0 | 2 | 7 | 5 | 16 | 14 | 20 | 19 | 22 | 15 | 11 | 11 | 8 | 3 | 1 | 0 | 1 | 0 | 1 |
| May ... | 0 | 0 | 0 | 0 | 1 | 0 | 2 | 2 | 4 | 3 | 7 | 12 | 16 | 31 | 24 | 14 | 6 | 11 | 3 | 1 | 1 | 0 | 0 | 0 |
| June ... | 0 | 0 | 0 | 2 | 0 | 3 | 3 | 6 | 6 | 13 | 15 | 10 | 16 | 33 | 13 | 11 | 4 | 6 | 3 | 1 | 0 | 0 | 0 | 0 |
| July ... | 2 | 0 | 1 | 0 | 0 | 3 | 1 | 5 | 6 | 10 | 13 | 15 | 16 | 18 | 12 | 16 | 14 | 7 | 3 | 1 | 0 | 0 | 0 | 0 |
| August ... | 0 | 0 | 0 | 0 | 1 | 1 | 3 | 0 | 2 | 2 | 7 | 9 | 8 | 19 | 17 | 9 | 3 | 7 | 1 | 1 | 3 | 0 | 0 | 1 |
| September ... | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 2 | 3 | 10 | 7 | 4 | 3 | 13 | 11 | 4 | 4 | 4 | 0 | 2 | 0 | 0 | 0 | 0 |
| | 2 | 0 | 2 | 2 | 2 | 7 | 11 | 22 | 26 | 54 | 63 | 70 | 78 | 136 | 92 | 65 | 42 | 43 | 13 | 7 | 4 | 1 | 0 | 2 |

III. SUMMER MONTHS: 'GREENWICH' TIME.

| | | | | | | | | | | | | | | | | | | | | | | | | |
|---------------|---|---|---|---|---|----|----|----|----|----|----|----|-----|----|----|----|----|----|---|---|---|---|---|---|
| April ... | 0 | 1 | 0 | 0 | 0 | 2 | 7 | 5 | 16 | 14 | 20 | 19 | 22 | 15 | 11 | 11 | 8 | 3 | 1 | 0 | 1 | 0 | 1 | 0 |
| May ... | 0 | 0 | 0 | 1 | 0 | 2 | 2 | 4 | 3 | 7 | 12 | 16 | 31 | 24 | 14 | 6 | 11 | 3 | 1 | 1 | 0 | 0 | 0 | 0 |
| June ... | 0 | 0 | 2 | 0 | 3 | 3 | 6 | 6 | 13 | 15 | 10 | 16 | 33 | 13 | 11 | 4 | 6 | 3 | 1 | 0 | 0 | 0 | 0 | 0 |
| July ... | 0 | 1 | 0 | 0 | 3 | 1 | 5 | 6 | 10 | 13 | 15 | 16 | 18 | 12 | 16 | 14 | 7 | 3 | 1 | 0 | 0 | 0 | 0 | 2 |
| August ... | 0 | 0 | 0 | 1 | 1 | 3 | 0 | 2 | 2 | 7 | 9 | 8 | 19 | 17 | 9 | 3 | 7 | 1 | 1 | 3 | 0 | 0 | 1 | 0 |
| September ... | 0 | 0 | 0 | 0 | 0 | 0 | 2 | 3 | 10 | 7 | 4 | 3 | 13 | 11 | 4 | 4 | 4 | 0 | 2 | 0 | 0 | 0 | 0 | 0 |
| | 0 | 2 | 2 | 2 | 7 | 11 | 22 | 26 | 54 | 63 | 70 | 78 | 136 | 92 | 65 | 42 | 43 | 13 | 7 | 4 | 1 | 0 | 2 | 2 |

1 p.m. (Greenwich time) in the summer months, *i.e.*, an hour earlier than in the winter months, may be explained on one of two assumptions:—

(1) That paroxysms actually occur earlier in the summer months than in the winter months, or

(2) That the change in habits of the patient, *e.g.*, getting up an hour earlier, has induced a corresponding change in the incidence of the paroxysms.

We incline to the latter view, and hence in constructing a graph of the paroxysms we combine the figures for the winter months (Greenwich time) with the figures for the summer months ('Summer' time).

If we divide the twenty-four hours into two periods, *viz.*, (a) 7 a.m. to 6.59 p.m., period of activity, and (b) 7 p.m. to 6.59 a.m., the period of rest, and determine the number of paroxysms which occur during each, we find, as is shewn in Table II, that 93.5 per cent. occur during the day and only 6.5 per cent. during the night.

TABLE II.

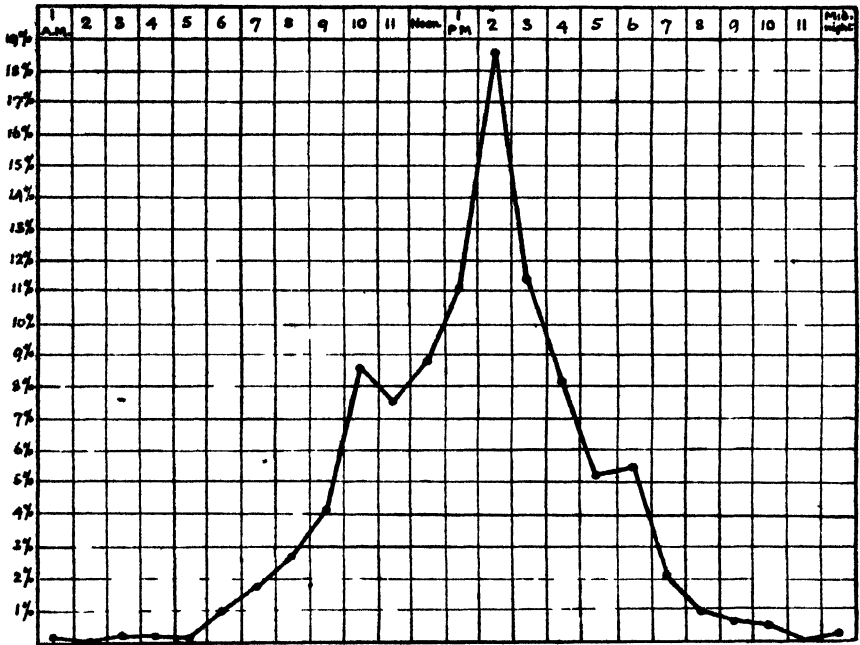
| Period. | Number of Paroxysms. | | |
|-----------------------------------|---|--------------------------------------|-------|
| | Winter Months (' Greenwich ' time) | Summer Months (' Summer ' time) | Total |
| 7 a.m. to 6.59 p.m. | 233 | 702 | 935 |
| 7 p.m. to 6.59 a.m. | 23 | 42 | 65 |

CONCLUSIONS

1. Over 90 per cent. of the paroxysms in simple tertian malaria occur during the hours of activity: in this series 7 a.m. to 6.59 p.m.

2. The maximum number of paroxysms under these conditions of activity occurred at 2 p.m.

3. Alteration of the period of activity by one hour, the result of the adoption of 'Summer time' produced a corresponding alteration in the time of incidence of the paroxysms.



GRAPH —Showing the time incidence of 1,000 simple tertian malaria paroxysms, 'Summer' time in operation

HAVE DIFFERENTIAL LEUCOCYTE COUNTS ANY VALUE ?

BY

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(Received for publication 6 January, 1921)

The figures obtained by us in differential leucocyte counts made from time to time on various blood films, mainly from cases of malaria, raised in our minds a doubt whether the procedure was of any value. We determined, therefore, to make a series of observations on normal and non-malarial bloods. As we proceeded it became evident that we must relinquish—temporarily at least—our initial aim, which was to determine whether the ‘normal values’ of the text-books were correct and substitute for it another, viz., the discovery of a method whereby a constant result (within certain limits) could be obtained from the blood of any individual. As, moreover, we did not know where the errors—which we felt certain existed—arose, whether, for instance, in the blood drop itself, in the making of the film, in the part of the film counted, in the process of counting, or in a combination of two or more of these possible sources of error, it was only gradually that order came out of our observations. In recording these observations, we have re-arranged them in the way that we consider will best illustrate some of the factors that must be taken into account.

Reflection shews that a differential leucocyte count can only be of value if the following conditions are satisfied.

1. That the portion of the film counted is of the same

composition as the rest of the film, or of the same composition as corresponding portions of the film. For example, if the count is made along an edge, the portion counted should at least give the same result as any other portion of the edge. In other words, we must be assured that the particular portion counted is representative of the whole film, or at least of definite zones of the film. All errors on this point could, of course, be eliminated by counting the whole film, but as a routine procedure this is impossible, as ordinary films may contain some 5,000 leucocytes, the counting of which would not only take several hours, but would be, as we shall show presently, a matter of extreme difficulty.

2.. That the blood which issues from the puncture is of the same composition as that in the vessels, *i.e.*, that various drops from various punctures of a single individual made at the same time are of identical composition.

These two fundamental conditions mean that the portion of the film counted must be representative of the blood on the film, and that the drop from which the film is made must be representative of the blood in the vessels; and it is clear that if they are not fulfilled the count is of no value.

3. That the 'normal values' of the text-books are correct, otherwise there is no standard with which any leucocyte count can be compared.

We may state at once that we have not been able to discover how these 'normal values' have been ascertained.

In order to find out how far these conditions hold good, we shall proceed to record a series of differential leucocyte counts made by ourselves.

METHOD EMPLOYED FOR MAKING BLOOD FILMS

Except when otherwise stated, the following technique was employed. A puncture was made with a surgical needle, and a drop of the blood issuing was taken on to a slide near one end. With the edge of another narrower slide (the spreader), held at an angle of about 45° , contact was made with the blood drop which by capillarity flowed along the angle between the two slides. The

spreader was then pushed steadily forward towards the other end of the slide, drawing the blood behind it so as to produce a thin uniform film (fig. 1), which was then fixed and stained with Leishman's stain in the usual way.

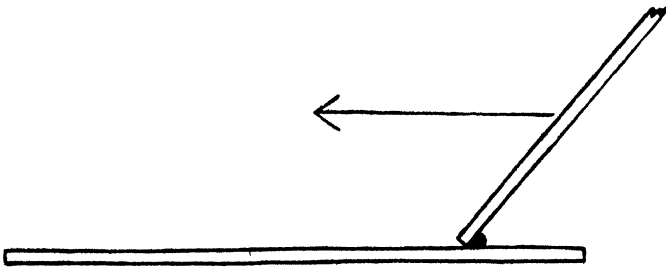


FIG 1. Illustrating method employed for making blood films

We will now consider some of the factors which may influence the count and be responsible for inconstant results

THE DISTRIBUTION OF LEUCOCYTES IN A FILM

Not very much or very precise information on this important question is to be found in the literature Rogers (1908), referring to films spread with a needle, writes, 'An excess of polynuclears and eosinophiles, and to a less extent of large mononuclears, will be found along either edge and in the distal tags marked PP (in fig 2),

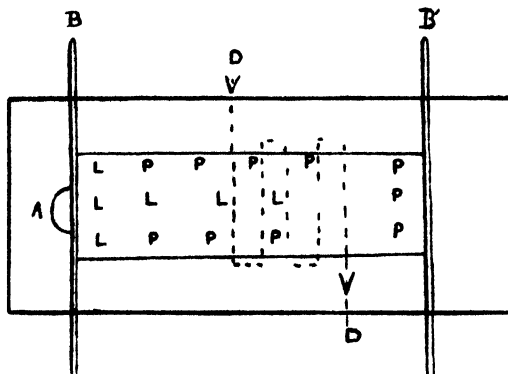


FIG 2 Methods of preparing blood films and the distribution of the various kinds of leucocytes in them (After Rogers)

while the lymphocytes will preponderate at the proximal end and in the central portions remote from either edge as indicated by the

letters LL. I have found, however, that by excluding the extreme ends of the films, and counting backwards and forwards across the intermediate portion between the dotted lines from edge to edge, very accurate percentages can be obtained by the enumeration of only 250 leucocytes whenever they are not present in considerable excess of the normal numbers the count can be readily carried out with a $\frac{1}{8}$ -inch or Zeiss D lens, by which means the time required after some practice to count the necessary 250 leucocytes in an ordinary film will not often exceed ten minutes, and thus become of easy clinical application.' Further, he writes, 'In order to ascertain the margin of error in counts of 250 to 500 leucocytes under different conditions, I worked out the percentages, after enumerating varying numbers in a long series of cases, with the following results. When the total number of leucocytes was approximately normal, counts of 250 and 500 respectively made in the manner just described gave variations of under 2 per cent in any of the four kinds; When, however, a leucocytosis was present the error from a similar count might amount to 2 or 3 per cent.' It must be noted that Rogers counts his leucocytes with a dry lens, and not with an oil immersion; his definition of a large mononuclear leucocyte is based solely on size, *i.e.*, one 'fully as large or larger than an average polynuclear.' Such a procedure is open to criticism, but we are not here concerned with this matter. Daniels and Newham (1911) state, 'To make a differential count of the leucocytes a dried film of a small but uncertain volume of blood is prepared and stained. All the leucocytes found in a systematic examination of a part of this film are counted, and the percentage of each different variety met with is thus ascertained. For accurate work not less than 500 should be counted, but for clinical purposes 200 will often suffice. The edges of the film where leucocytes are most numerous should not be included in the enumeration.'

While Rogers recommends transverse counts, Daniels and Newham recommend counts of central areas. Neither has, however, demonstrated that the particular method which he favours gives results which most closely approximate the actual result which would be obtained by counting all the leucocytes in the film: and, moreover, it is clear that if different results are to be obtained by

different methods of counting, then it is useless to compare figures obtained by the method recommended by Rogers with those obtained by the method recommended by Daniels and Newham.

These points appear to us to be among the first which require examination.

We have purposely avoided any attempt to classify the leucocytes into their various sub-groups, and have confined ourselves solely to classifying them as mononuclear and polymorphonuclear.

The figures given in the following tables indicate the percentage of the total leucocytes constituted by the mononuclear variety—in short, the mononuclear percentage.

EXAMINATION OF THE WHOLE OF A SMALL FILM

The whole of two small films was examined and the mononuclear percentage determined for the various areas into which they were ruled, as is shewn in figs. 3 and 4.

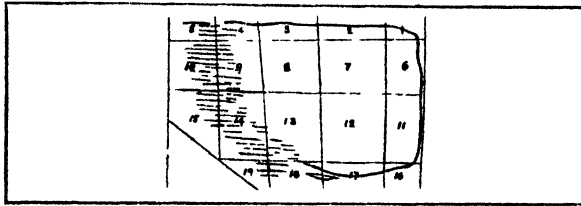


FIG. 3 Shewing areas into which the blood film was ruled.

EXPERIMENT I.

- a. Blood of normal adult (S.M.) who has been in the tropics and who has had malaria.
- b. Blood taken from dorsum of finger.
- c. Counts made by a single observer.

TABLE I.

Shewing distribution of leucocytes in a film.

| Various areas examined | Total number of leucocytes. | Mononuclear percentage |
|--|-----------------------------|------------------------|
| 1. Central areas (7, 8, 12, 13) | 185 | 45.0 |
| „ including part 'tongue' areas (7, 8, 9, 12, 13, 14) | 374 | 41.0 |
| 2. Transverse areas (2, 7, 12, 17, 3, 8, 13, 18) | 375 | 39.0 |
| 3. Edge areas (1-5, 16-18) | 162 | 29.6 |
| 4. 'Tongue' areas (5, 10, 15, 19) | 972 | 25.7 |
| 5. Total film 1-19 | 1660 | 30.6 |

EXPERIMENT IA.

a—c as in Experiment I.

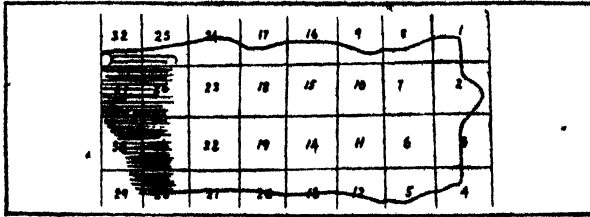


FIG. 4. Shewing areas into which the blood film was ruled.

TABLE IA.

Distribution of leucocytes in a film.

| Various areas examined | Total number of leucocytes | Mononuclear percentage |
|---|----------------------------|------------------------|
| 1. Central areas (6, 7, 10, 11, 14, 15, 18, 19, 22, 23, 26, 27) . | 274 | 48.2 |
| 2. Transverse areas (5-28) | 547 | 39.8 |
| 3. Edge areas (1, 8, 9, 16, 17, 24, 25, 4, 5, 12, 13, 20, 21, 28) | 290. | 32.8 |
| 4. ' Tongue ' areas (29-32) | 622 | 20.4 |
| 5. Total film 1 to 32 | 1252 | 29.6 |

These observations shew :—

1. That the mononuclear percentage is highest in the central areas of a film and lowest in the tongue areas; the edge areas give a figure which is intermediate. .

2. That the mononuclear percentage found in the edge areas approximates most closely to that found in the total film.

EXAMINATION OF DIFFERENT AREAS OF ORDINARY (LARGE) FILMS

In this series of observations on ordinary films, no attempt was made to count all the leucocytes in the film, but a definite number only were examined in certain areas which in this and in the succeeding observations may be defined as follows:—

Edge areas. Areas bounded by the edges of the film and imaginary lines parallel to them at a distance from the edges equal to that of the diameter of the microscope field given by a $1/12$ oil immersion lens and a 4 ocular.

Central areas. The part of the film lying between the edge areas.

Transverse areas. Composite areas embracing the former areas, the counts being made by examining the slide methodically from edge to edge.

In all cases, as shewn in fig. 5, two lines were drawn across the film excluding the commencement and tongues of the film respectively: all the counts were made between these two lines.

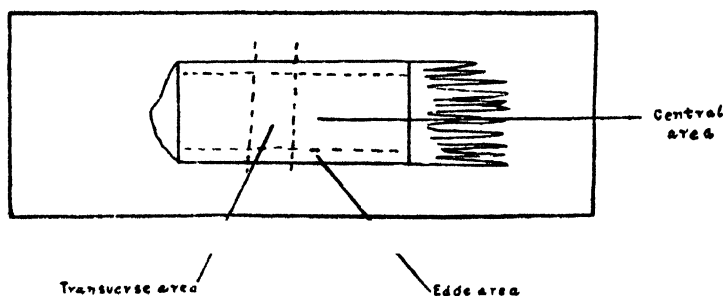


FIG 5 Shewing various areas of a blood film.

EXPERIMENT II.

- a. Blood of 4 patients: dysentery (1), myalgia (1), and trench fever (2).
- b. Blood from ear spread with a needle.
- c. A film was made from each of the patients on a number of consecutive days, as shewn in the table, making in all 34 films.
- d. A count made by a single observer.
- e. 250 leucocytes counted in each area.

TABLE II.

Shewing results obtained by counting 250 leucocytes in different areas of the same films.

| Case | Days | Central areas | Transverse areas | Edge areas | Range |
|------------------|------|---------------|------------------|------------|-------|
| Dysentery ... | 1 | 62 | 57 | 54 | 8 |
| | 2 | 56 | 55 | 53 | 3 |
| | 3 | 65 | 54 | 61 | 11 |
| | 4 | 58 | 53 | 54 | 5 |
| | 5 | 60 | 59 | 42 | 28 |
| | 6 | 60 | 56 | 46 | 14 |
| | 7 | 72 | 58 | 60 | 14 |
| | 8 | 64 | 66 | 66 | 22 |
| Myalgia ... | 1 | 45 | 43 | 38 | 7 |
| | 2 | 41 | 58 | 46 | 17 |
| | 3 | 59 | 53 | 41 | 18 |
| | 4 | 51 | 64 | 51 | 13 |
| | 5 | 62 | 52 | 50 | 12 |
| | 6 | 42 | 46 | 38 | 8 |
| | 7 | 56 | 47 | 43 | 13 |
| | 8 | 47 | 50 | 49 | 23 |
| Trench fever ... | 1 | 53 | 45 | 50 | 8 |
| | 2 | 63 | 47 | 43 | 20 |
| | 3 | 52 | 48 | 44 | 8 |
| | 4 | 43 | 45 | 35 | 10 |
| | 5 | 56 | 46 | 44 | 12 |
| | 6 | 59 | 52 | 34 | 25 |
| | 7 | 47 | 47 | 42 | 5 |
| Trench fever .. | 1 | 47 | 47 | 39 | 8 |
| | 2 | 43 | 42 | 41 | 2 |
| | 3 | 58 | 49 | 50 | 9 |
| | 4 | 58 | 44 | 43 | 19 |
| | 5 | 63 | 60 | 58 | 5 |
| | 6 | 48 | 52 | 38 | 10 |
| | 7 | 55 | 56 | 52 | 4 |
| | 8 | 53 | 46 | 46 | 7 |
| | 9 | 35 | 42 | 40 | 7 |
| | 10 | 35 | 20 | 26 | 15 |
| | 11 | 38 | 46 | 42 | 8 |

It is seen from Table II that the central areas gave a higher mononuclear percentage than the edge areas of the same film in twenty-eight of thirty-four instances, an equal percentage in one instance, and a lower percentage in five. In about half the cases the mononuclear percentage of the transverse area was intermediate between those for the central and edge areas of the same film.

EXPERIMENT III.

- a. Blood of normal adult.
- b. Blood taken from ear and spread with a needle.
- c. A film was made on each of five consecutive days.
- d. Count made by a single observer.
- e. 1,000 leucocytes counted in each area.

TABLE III.

Shewing results obtained by counting 1000 leucocytes in different areas of the same films.

| Days | Central areas | Transverse areas | Edge areas | Range |
|------|---------------|------------------|------------|-------|
| 1 | 47 | 46 | 40 | 7 |
| 2 | 65 | 61 | 54 | 11 |
| 3 | 61 | ... | 47 | 14 |
| 4 | 51 | ... | 44 | 7 |
| 5 | 52* | ... | 42* | 10 |

* 500 leucocytes only counted.

In this series of observations the highest mononuclear percentage was found in the central areas and the lowest in the edge areas in each case; the transverse areas were intermediate.

Consideration of the results of these earlier experiments shews that in a blood film the distribution of leucocytes, in respect of mononuclear percentage, is irregular. The mononuclear percentage is lowest in the tongue areas and increases as we pass from the edge areas to the transverse areas, and reaches its maximum in the central areas; of these various areas the edge areas give a mononuclear percentage which approximates most closely to that of the total film. This fact is clearly brought out in Experiments I and IA, where all the leucocytes in the two films were counted; it is also shown in Experiment III, where 1,000 leucocytes were counted in the various areas; but in Experiment II, where only 250 leucocytes were counted, the result was to some extent contradictory. It follows from this that for purposes of comparison, counts of edge areas only, or of transverse areas only, can be used. As edge areas give a mononuclear percentage which approximates most closely to that of the total film, and as they are more easily and rapidly counted than transverse or central areas, it appears desirable to confine the counts to these areas.

In view of the fact that counts of 250 fail to demonstrate the fact that the mononuclear percentage of edge areas is less than that of central or transverse areas, they are of no value. Further evidence of the inconstant results obtained by 250 counts is given by the following experiments.

EXPERIMENT IV.

- a. Blood of healthy adult C., who had never been out of England.
- b. Blood taken from dorsum of finger.
- c. Films made from 1st and 5th drops issuing from puncture at 12.30 p.m. and also at 2.30 p.m. for five consecutive days.
- d. 250 leucocytes counted by each of six observers.
 - i. Edge counts.
 - ii. Transverse counts.

TABLE IV.

Shewing results obtained by six observers each counting 250 leucocytes in edge and transverse areas of each of a number of films made at different times from one individual.

| Days | Times | Drop | EDGE COUNTS | | | | | TRANSVERSE COUNTS | | | | |
|------|-------|------|---------------------------------|---------|---------|-------|---------------------------------|---------------------------------|---------|---------|-------|----------------------------------|
| | | | Average counts of six observers | Maximum | Minimum | Range | Greatest variation from average | Average counts of six observers | Maximum | Minimum | Range | Greatest variations from average |
| 1 | 12.30 | 1st | 39 | 42 | 35 | 7 | 4 | 50 | 54 | 38 | 16 | 12 |
| | | 5th | 41 | 45 | 37 | 8 | 4 | 43 | 48 | 39 | 9 | 5 |
| | 2.30 | 1st | 31 | 33 | 30 | 3 | 2 | 43 | 48 | 38 | 10 | 5 |
| | | 5th | 29 | 32 | 26 | 6 | 3 | 40 | 44 | 38 | 6 | 4 |
| 2 | 12.30 | 1st | 28 | 30 | 26 | 4 | 2 | 44 | 52 | 40 | 12 | 8 |
| | | 5th | 28 | 30 | 27 | 3 | 2 | 44 | 51 | 42 | 9 | 7 |
| | 2.30 | 1st | 29 | 34 | 26 | 8 | 5 | 40 | 46 | 35 | 11 | 6 |
| | | 5th | 32 | 36 | 26 | 10 | 6 | 39 | 43 | 36 | 7 | 4 |
| 3 | 12.30 | 1st | 41 | 43 | 37 | 6 | 4 | 51 | 62 | 46 | 16 | 11 |
| | | 5th | 29 | 32 | 27 | 5 | 3 | 48 | 53 | 46 | 7 | 5 |
| | 2.30 | 1st | 30 | 33 | 28 | 5 | 3 | 44 | 49 | 39 | 10 | 5 |
| | | 5th | 36 | 45 | 32 | 13 | 9 | 49 | 55 | 39 | 16 | 10 |
| 4 | 12.30 | 1st | 38 | 39 | 30 | 3 | 2 | 43 | 46 | 37 | 9 | 6 |
| | | 5th | 40 | 44 | 38 | 6 | 4 | 43 | 48 | 37 | 11 | 6 |
| | 2.30 | 1st | 38 | 39 | 37 | 2 | 1 | 47 | 56 | 40 | 16 | 9 |
| | | 5th | 30 | 31 | 30 | 1 | 1 | 48 | 52 | 39 | 13 | 9 |
| 5 | 12.30 | 1st | 44 | 46 | 42 | 4 | 2 | 49 | 56 | 46 | 10 | 7 |
| | | 5th | 37 | 38 | 34 | 4 | 3 | 50 | 56 | 40 | 16 | 10 |
| | 2.30 | 1st | 39 | 40 | 37 | 3 | 2 | 51 | 53 | 48 | 7 | 4 |
| | | 5th | 35 | 37 | 32 | 5 | 3 | 45 | 47 | 42 | 5 | 3 |

EXPERIMENT IVa.

a. Blood of healthy adult M., who had never been out of England.

b-d. As in Experiment IV.

TABLE IVa.

Shewing the results obtained by six observers each counting 250 leucocytes in edge and transverse areas of each of a number of films made at different times from an individual

| Days | Times | Drop | EDGE COUNTS | | | | | TRANSVERSE COUNTS | | | | |
|------|-------|------|---------------------------------|---------|---------|-------|---------------------------------|---------------------------------|---------|---------|-------|---------------------------------|
| | | | Average counts of six observers | Maximum | Minimum | Range | Greatest variation from average | Average counts of six observers | Maximum | Minimum | Range | Greatest variation from average |
| 1 | 12.30 | 1st | 34 | 37 | 32 | 5 | 3 | 47 | 51 | 42 | 9 | 5 |
| | | 5th | 38 | 40 | 37 | 3 | 2 | 51 | 57 | 46 | 11 | 6 |
| | 2.30 | 1st | 41 | 45 | 39 | 6 | 4 | 47 | 49 | 41 | 8 | 6 |
| | | 5th | 34 | 36 | 31 | 5 | 3 | 49 | 57 | 40 | 17 | 9 |
| 2 | 12.30 | 1st | 42 | 45 | 40 | 5 | 3 | 47 | 50 | 40 | 10 | 7 |
| | | 5th | 39 | 43 | 34 | 9 | 5 | 46 | 52 | 40 | 12 | 6 |
| | 2.30 | 1st | 45 | 47 | 42 | 5 | 2 | 48 | 56 | 44 | 12 | 8 |
| | | 5th | 37 | 38 | 35 | 3 | 2 | 49 | 54 | 42 | 12 | 7 |
| 3 | 12.30 | 1st | 42 | 44 | 38 | 6 | 4 | 45 | 48 | 35 | 13 | 10 |
| | | 5th | 38 | 40 | 36 | 4 | 2 | 51 | 56 | 48 | 8 | 5 |
| | 2.30 | 1st | 37 | 41 | 34 | 7 | 4 | 38 | 44 | 35 | 9 | 6 |
| | | 5th | 39 | 43 | 37 | 6 | 4 | 47 | 52 | 40 | 12 | 7 |
| 4 | 12.30 | 1st | 40 | 46 | 35 | 11 | 6 | 54 | 58 | 50 | 8 | 4 |
| | | 5th | 41 | 44 | 36 | 8 | 3 | 50 | 57 | 43 | 14 | 7 |
| | 2.30 | 1st | 41 | 44 | 35 | 9 | 6 | 55 | 60 | 52 | 8 | 5 |
| | | 5th | 38 | 40 | 35 | 5 | 3 | 48 | 50 | 46 | 4 | 2 |
| 5 | 12.30 | 1st | 43 | 46 | 39 | 7 | 4 | 51 | 57 | 47 | 10 | 6 |
| | | 5th | 37 | 38 | 34 | 4 | 3 | 50 | 52 | 46 | 6 | 4 |
| | 2.30 | 1st | 28 | 30 | 27 | 3 | 2 | 42 | 46 | 40 | 6 | 4 |
| | | 5th | 40 | 42 | 38 | 4 | 2 | 50 | 54 | 48 | 6 | 4 |

If we compare the results of the 250 counts obtained by each of the six observers for the same areas of the same films, we find very great variations. In the case of the edge areas the greatest range between the counts of the six observers for the same films were in the two cases respectively 13 and 11, and the maximum variations from the average of the six counts 9 and 6: in the transverse areas the ranges were respectively 16 and 17, and the maximum variation from the average 12 and 10. These variations are so great that it is obvious that they must be explained before proceeding any further.

The number of leucocytes comprising the individual count of each observer was only 250; it is hardly possible that in any film two observers counted exactly the same leucocytes, in fact, in some instances the 250 leucocytes counted by one observer were entirely different from those counted by another. For example, one observer examines one edge of the film and another the other edge, or if they both examine the same edge they begin at different ends; this applies also in the case of transverse counts. It is possible, therefore, that the ranges in the individual counts may be due to the fact that different leucocytes were examined.

Another possibility is that the same leucocytes were differently interpreted by different individuals. Finally, the differences may be the result of a combination of these two factors.

In order to investigate the problem, minute blood films of the same two individuals were made, and all the leucocytes present in them were examined by a number of different observers. To ensure that every leucocyte was counted once, and once only, a small square aperture was cut in a disc of gelatine, which was inserted into the ocular; by means of a mechanical stage the whole film was then passed in regular sequence under the square field.

EXPERIMENT V.

- a. Blood of healthy adult C., who had never been out of England.
- b. Blood taken from dorsum of finger.
- c. Minute blood films made from 1st and 5th drops issuing from puncture at 12.30 p.m. and 2.30 p.m. on two days.
- d. The whole of each film counted by five observers.

EXPERIMENT VA.

- a. Blood of healthy adult M., who had never been out of England.
- b—d. As in Experiment V.

TABLES V AND VI.

| Case | Date | Time | Drop No. | | Results obtained by the five observers | | | | | Average of results obtained by five observers | Range | Greatest variation from the average |
|------|---------|------------|----------|------|--|------|------|------|------|---|-------------------------|-------------------------------------|
| | | | | | M. | C. | S. | B. | Co. | | | |
| C | Jan. 10 | p.m. 12.30 | 1st | T.L. | 205 | 205 | 196 | 203 | 217 | 205 | 21 (10.2%) [*] | 3.2 |
| | | | | M. % | 43.9 | 45.4 | 42.8 | 39.9 | 43.7 | 43.1 | 5.5 | |
| | | 5th | | T.L. | 247 | 248 | 242 | 257 | 257 | 250 | 15 (6%) | 2.2 |
| | | | | M. % | 48.6 | 50.0 | 47.9 | 45.9 | 47.9 | 48.1 | 4.1 | |
| | | 2.30 | 1st | T.L. | 465 | 459 | 429 | 455 | 460 | 453.6 | 36 (7.9%) | 1.3 |
| | | | | M. % | 33.8 | 32.3 | 32.1 | 31.8 | 34.1 | 32.8 | 2.3 | |
| | Jan. 15 | 5th | | T.L. | 513 | 519 | 525 | 521 | 522 | 520 | 12 (2.3%) | 1.2 |
| | | | | M. % | 34.1 | 34.3 | 34.8 | 33.2 | 35.6 | 34.4 | 2.4 | |
| | | 12.30 | 1st | T.L. | 167 | 162 | 166 | 164 | 170 | 165.8 | 8 (4.7%) | 0.8 |
| | | | | M. % | 40.7 | 42.0 | 42.1 | 40.8 | 41.1 | 41.3 | 1.4 | |
| | | 5th | | T.L. | 158 | 155 | 161 | 153 | 162 | 157.8 | 9 (5.7%) | 1.8 |
| | | | | M. % | 43.7 | 45.2 | 42.2 | 45.7 | 43.2 | 44.0 | 3.5 | |
| M | Jan. 10 | 2.30 | 1st | T.L. | 203 | 205 | 186 | 191 | 205 | 198 | 19 (9.5%) | 3.8 |
| | | | | M. % | 37.4 | 38.1 | 37.1 | 36.6 | 32.6 | 36.4 | 5.5 | |
| | | 5th | | T.L. | 264 | 269 | 254 | 254 | 279 | 264 | 25 (9.5%) | 0.9 |
| | | | | M. % | 31.4 | 33.1 | 32.6 | 32.0 | 32.2 | 32.3 | 1.7 | |
| | Jan. 15 | p.m. 12.30 | 1st | T.L. | 262 | 261 | 251 | 271 | 261 | 261.2 | 20 (7.6%) | 1.4 |
| | | | | M. % | 37.0 | 38.4 | 38.9 | 36.1 | 36.4 | 37.5 | 2.8 | |
| | | 5th | | T.L. | 103 | 101 | 105 | 105 | 104 | 103.6 | 4 (3.8%) | 2.3 |
| | | | | M. % | 37.9 | 40.6 | 41.9 | 39.0 | 38.4 | 39.6 | 4.0 | |
| | | 2.30 | 1st | T.L. | 208 | 212 | 208 | 216 | 205 | 209.8 | 11 (5.2%) | 1.1 |
| | | | | M. % | 40.8 | 40.1 | 39.4 | 39.3 | 39.0 | 39.7 | 1.8 | |
| M | Jan. 10 | 5th | | T.L. | 555 | 559 | 565 | 522 | 563 | 552.8 | 43 (7.7) | 0.5 |
| | | | | M. % | 34.6 | 35.1 | 35.0 | 34.8 | 35.5 | 35.0 | 0.9 | |
| | Jan. 15 | 12.30 | 1st | T.L. | 148 | 142 | 144 | 142 | 144 | 144 | 6 (4.2%) | 0.9 |
| | | | | M. % | 36.5 | 35.9 | 35.5 | 35.2 | 34.7 | 35.6 | 1.8 | |
| | | 5th | | T.L. | 103 | 97 | 90 | 91 | 96 | 95.4 | 13 (13.6%) | 4.0 |
| | | | | M. % | 41.7 | 46.3 | 45.5 | 39.5 | 38.5 | 42.3 | 7.8 | |
| | 2.30 | 1st | | T.L. | 150 | 147 | 142 | 144 | 149 | 146.4 | 8 (5.5%) | 1.4 |
| | | | | M. % | 26.0 | 27.3 | 28.8 | 26.3 | 28.8 | 27.4 | 2.8 | |
| M | Jan. 15 | 5th | | T.L. | 138 | 134 | 127 | 121 | 120 | 128 | 18 (14.1%) | 1.6 |
| | | | | M. % | 21.7 | 23.9 | 23.6 | 20.8 | 21.6 | 22.3 | 3.1 | |

* The figures in brackets represent the range expressed as a percentage of the actual total (average of five observers).

T.L. = Total Leucocytes in film. M. % = Mononuclear percentage.

In none of the sixteen films in Experiments V and VA did more than two observers agree as to the total number of leucocytes present. The range between highest and lowest total count varied in the case of the different films from 2·3 to 14·1 per cent. of the actual total (average of five observers) for the corresponding film.

This failure to agree on the exact number of leucocytes in a film may at first sight be rather surprising, but the procedure is not so simple as it may appear. The following seem to us to be some of the causes most likely to produce the discrepancy :—

1. Failure to pass the whole film under review once, and once only, due to defective action of the mechanical stage, which would result in portions of the film being either omitted or re-observed.
2. The end portion of a film consists of small tongue-like prolongations and minute islands, and possibly some of the latter escaped the observation of certain of the observers.
3. In the process of making the minute films, a number of leucocytes are damaged, and fragments which may be omitted from the counts of one observer as unrecognisable may be sufficiently identified by another for inclusion. Or again, if a leucocyte is split into two approximately equal portions, which become separated one from the other, the fractions may be both omitted by one observer or counted as two leucocytes by another.

Whatever the reasons for the discrepancy may be, the fact remains that we did not succeed in accomplishing what we hoped to do, viz., making certain that an opinion was passed once, and once only, on every leucocyte in a film by each of the five observers. Having failed to do this, we are not in a position to ascertain absolutely the amount of error arising from the personal factor of interpretation. However, as the figures given by each observer for the total leucocyte count are comparatively close, we can reasonably infer that whatever the actual totals in the various films were, a very large percentage of the leucocytes in each film had been examined by each observer once, and once only, and that, therefore, the figure for the mononuclear percentage was obtained by each observer from an examination of practically the same leucocytes.

It will be seen from Table V that the maximum range in mononuclear percentage obtained by the five observers examining the same film were in the two cases respectively 5·5 and 7·8, while

the maximum variations from the actual mononuclear percentage (average of the five observers) were 3·8 and 4·0.

In view of the fact mentioned above that the different observers were not dealing with exactly the same leucocytes, these discrepancies cannot be explained altogether by error of interpretation, although it is probable that the personal factor is partly responsible. It must, however, be recognised that the personal factor of interpretation is subjected to a much more severe (possibly hardly fair) test in the case of total counts of minute films than in counts of selected areas of ordinary (large) films. It will be remembered that in dealing with ordinary films, the two ends (commencement and tongues) were ruled off and excluded from the counts, whereas in the case of total counts of the minute films, both commencement and tongues of the film are necessarily included: it is at these extremities of the film that the greatest difficulty of interpretation is experienced, because the commencement of the film is usually its thickest portion and the tongue areas contain the greatest proportion of damaged leucocytes.

Notwithstanding these considerations, the maximum variation from the actual mononuclear percentage obtained by any of the observers in the sixteen minute films is only 4, which is relatively small when compared with 9 for the forty edge counts and with 12 for the forty transverse counts in Experiment IV and IVa, showing clearly that the more carefully we arrange that the different observers examine the same leucocyte the more closely do the results obtained by the different observers approximate.

We infer from this, that the errors arising from differences of interpretation are relatively small, and can in no way explain the large differences obtained by the various individuals in examining the edge and transverse areas of the same films in Experiments IV and IVa; we are, therefore, forced to conclude that the explanation of the discrepancies is that they are due in the main to the fact that the different observers examined different leucocytes. In other words, it appears that the distribution of leucocytes as regards mononuclear percentage is so irregular in both the edge and transverse areas, that samples of 250 are far too small to give constant results for the same edge or transverse areas.

We next decided to ascertain whether more constant results could

be obtained from large counts, *e.g.*, 500, 1,000, 2,000, 4,000, etc. As there is only a limited number of leucocytes in the edge areas or transverse areas of any film, it is clear that a number of films must be used for such large counts. This may be done, provided we are certain that the constitution of the blood on the various films is identical. A difficulty at once arises, because we have no knowledge that various drops issuing from the same puncture are identical in composition, and still less that drops issuing from different punctures are of the same composition. However, for the purpose of the next experiment, we have assumed that when blood is issuing freely from a puncture, successive drops are of the same constitution.

EXPERIMENT USING SUCCESSIVE DROPS OF BLOOD FLOWING FREELY FROM SAME PUNCTURE

EXPERIMENT VI.

- a. Blood of normal adult S.M., who has been in tropics and who has had malaria.
- b. Blood of dorsum of finger.
- c. Deep puncture made and free flow of blood obtained.
- d. Films made from drops 7-10, 13-16, 19-22, and 25-28 (sixteen films).
- e. Four observers each count a separate film in each set.
- f. 500 leucocytes counted by each observer.
- g. Edge areas (excluding tongues).

EXPERIMENT VIA.

- a. Blood of normal adult Y., who has been in tropics and who has had malaria.
- b.-g. As in Experiment VI.

Tables VI and VIA shew that in counts of edge areas of films made from successive drops of blood freely flowing from the same puncture, the range obtained when 500 leucocytes are counted was in the two cases M. and Y. respectively 11 and 10, and when 1,000 were counted 6.5 and 5.7. The greatest variation from the figure obtained by counting 8,000 leucocytes was in the 500 counts 8 and 6 respectively in the two cases, and in the 1,000 counts 4 and 3.25. If we assume that the successive drops of blood flowing freely from the same puncture are of constant composition, then from the above experiment it follows that if only 500 leucocytes are counted, the result obtained may be as much as 8 above or 8 below the 'true figure' obtained by the

8,000 counts, and if 1,000 leucocytes are counted the result may be as much as 4 above and 4 below the 'true figure.' Consequently, differences as great as 16 obtained in edge counts of 500 leucocytes and as great as 8 in edge counts of 1,000 leucocytes do not necessarily mean that the mononuclear percentage in the various bloods concerned is actually different.

These preliminary experiments which deal solely with attempts to determine the mononuclear percentage of the blood of normal persons suffice, we think, to shew that considerable difficulties surround what has hitherto been regarded as a simple matter.

TABLE VI.

Experiment with successive drops of free flowing blood from same puncture to shew variation obtained by counts of 500 and 1000 respectively.

| No. of drop | Observer | Results of 500 counts | Range of eight counts of 500 | Results of 1000 counts | Range of eight counts of 1000 | Results of 8000 counts | Maximum variation from result of 8000 count obtained by | |
|-------------|----------|-----------------------|------------------------------|------------------------|-------------------------------|------------------------|---|------------|
| | | | | | | | 500 count | 1000 count |
| 7 | S. | 32 | 11 | 32.5 | 6.5 | 31 | 8 | 4 |
| 8 | M. | 33 | | | | | | |
| 9 | C. | 29 | | 30.0 | | | | |
| 10 | Y. | 31 | | | | | | |
| 13 | S. | 31 | | 35.0 | | | | |
| 14 | M. | 39 | | | | | | |
| 15 | C. | 32 | 10 | 30.0 | | | | |
| 16 | Y. | 28 | | | | | | |
| 19 | S. | 32 | | 33.5 | | | | |
| 20 | M. | 15 | | | | | | |
| 21 | C. | 31 | | 29.5 | | | | |
| 22 | Y. | 28 | | | | | | |
| 25 | S. | 25 | | 28.5 | | | | |
| 26 | M. | 32 | | 29.0 | | | | |
| 27 | C. | 29 | | | | | | |
| 28 | Y. | 29 | | | | | | |

TABLE VIA.

Experiment with successive drops of free flowing blood from same puncture to show variation obtained by counts of 500 and 1000 respectively.

| No. of drop | Observer | Results of 500 counts | Range of eight counts of 500 | Results of 1000 counts | Range of eight counts of 1000 | Results of 8000 count, | Maximum variation from result of 8000 count obtained by | |
|-------------|----------|-----------------------|------------------------------|------------------------|-------------------------------|------------------------|---|------------|
| | | | | | | | 500 count | 1000 count |
| 7 | S. | 50.0 | 8 | 54.0 | 5.75 | 52 | 6 | 3.25 |
| 8 | M. | 58.0 | | 53.25 | | | | |
| 9 | C. | 53.5 | | 52.0 | | | | |
| 10 | Y. | 53.0 | | 51.0 | | | | |
| 13 | S. | 52.0 | | 51.5 | | | | |
| 14 | M. | 52.0 | 10 | 55.25 | | | | |
| 15 | C. | 51.0 | | 49.5 | | | | |
| 16 | Y. | 51 | | 51.5 | | | | |
| 19 | S. | 51 | | 51.5 | | | | |
| 20 | M. | 52.0 | | 51.5 | | | | |
| 21 | C. | 52.5 | | 51.5 | | | | |
| 22 | Y. | 58.0 | | 51.5 | | | | |
| 25 | S. | 49.0 | | 51.5 | | | | |
| 26 | M. | 50.0 | | 51.5 | | | | |
| 27 | C. | 55.0 | | 51.5 | | | | |
| 28 | Y. | 48.0 | | 51.5 | | | | |

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PRELIMINARY NOTE ON THE
DEVELOPMENT OF THE LARVAE OF
DIROFILARIA IMMITIS IN DOG FLEAS
CTENOCEPHALUS FELIS AND *CANIS*

BY

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Dirofilaria immitis (Blanchard 1895) is a parasite commonly found in dogs in and around Townsville, and a number of the animals succumb to the infection with this parasite under similar clinical symptoms to those described from other parts of the world. Microfilariae can usually be seen in the peripheral blood of the infected animals at any hour of the day or night.

The transmission of *Dirofilaria immitis* by mosquitoes has been definitely proved by several writers, and the parasite undergoes its full development of cycle in mosquitoes belonging to such different genera as *Stegomyia*, *Anopheles* and *Culex*.

The fact that this parasite does not require a definite genus of mosquito for the successful completion of its life history, as definitely proved for the majority of protozoal infections, suggested further investigations to ascertain whether other ectoparasites of dogs, such as fleas, offer suitable conditions for the development of the larvae of *Dirofilaria immitis*, when taken up in the infected blood by the fleas.

T. L. Bancroft in Australia, and others in different parts of the

world, have suspected *Pulex serraticeps*, but were unable to trace further development of the larvae after the blood containing them was digested.

Fleas were collected from dogs known to harbour the parasite in their peripheral blood, and were dissected in the usual way. As a rule, *Ctenocephalus canis* and *felis* occurred in about the same proportion, but at other times the number of *C. felis* much preponderated. The terminal segment of the flea was snipped on both sides, and by gentle traction the proventriculus, mid-gut and hind-gut with the Malpighian tubes were removed as a whole.

The alimentary canal was teased out on a slide in a small quantity of saline, and the specimen thus obtained fixed before drying in a fixing solution containing equal parts of concentrated picric acid and mercuric chloride, to which acetic acid was added to make it up to a 4 per cent. solution. The films were fixed for a few minutes in this solution, hardened in alcohol of increasing strength and afterwards stained with Ehrlich's haematoxylin. A number of other stains were experimented with, but the results were in no case superior to that obtained with haematoxylin. We were thus able to obtain 'wet' films, which, on microscopical examination, showed structural details not seen in dried specimens.

Whilst dissecting fleas, it was noticed on several occasions that when teasing out the alimentary canal large nematodes escaped from the body cavity which were actively motile, and could not be differentiated from mature filarial larvae such as seen in the infected mosquito.

Fleas collected from an infected dog which contained fresh blood, more or less altered, in their mid-gut, showed, as a rule, filarial larvae in a varying quantity resembling closely the forms seen in the peripheral blood of the dog. In addition, forms were seen with a slightly broadened posterior end (measuring in the stained specimens 6μ to 8μ), whereas the diameter at the anterior end measured about 4μ to 5μ . In many instances the Malpighian tubes of 'infected' fleas contained parasites further developed: small stumpy forms about 90μ to 120μ long and 14μ to 16μ broad with a pointed tail end, longer forms up to 250μ and more in length and up to 30μ in width; in addition, many intermediate stages were seen on different occasions. The large, free

forms up to 600μ in length and 20μ to 30μ wide were found now and again free in the body cavity of the flea.

Our experience has thus shown that the larvae of *Dirofilaria immitis* undergo a complete developmental cycle, analogous to that in the mosquito (*Stegomyia fasciata* was used in our experiments) in the dog fleas, *Ctenocephalus canis* and *felis*. The larvae enter the flea with the blood and obtain access to the Malpighian tubes, where the further development takes place. When nearly mature they leave the Malpighian tubes, and are found free in the body cavity.

It was noted that the degree of development depended greatly on the external temperature. During the hot season fully-developed larvae were frequently found, whereas during our cool months the larvae hardly ever developed beyond the early stages, and, as a rule, only blood forms with the broadened posterior end were discovered. However, in addition to temperature, other factors which could not be determined seemed to influence the development.

On several occasions fleas collected from one dog were heavily infected, whereas fleas collected from another dog living in the same surroundings, with approximately the same number of parasites in the peripheral blood, showed only scanty parasites. Even fleas collected from the same dog showed great variation in the infection. The fleas of the same animal would show during one week a large number of forms in all stages, whereas the following week only blood, and perhaps early forms, could be found in the dissections.

In our experience, development of the filarial larvae occurred most often in the female flea; whereas in the male flea, only now and again were early developmental stages discovered.

The mode of transmission was considered, and it was thought possible that under normal conditions the flea would be crushed, and the actively motile larvae thus freed would penetrate through the unbroken skin and reach the blood stream in analogy with the mechanism of *Aegylosoma* infection.

In order to ascertain the correctness of the surmise, fleas were crushed over the shaved skin of young puppies which had been moistened with saline, and after an interval of from five to twenty minutes the part of the skin was excised and serial sections prepared for microscopical examination.

In one instance only out of seven experiments one mature larva was discovered in three consecutive serial sections with the anterior part (about 30 μ of the larva) embedded in the subcutaneous tissue, the remaining part adhering to the outer skin. The larva had, without doubt, penetrated through the unbroken skin.

SUMMARY

The experiments carried out have proved that the larvae of *Dirofilaria immitis* can undergo a complete developmental cycle in the dog fleas, *Ctenocephalus felis* and *canis*. A mature larva on one occasion was found to have penetrated the unbroken skin of a puppy, making its way into the subcutaneous tissue.

INDEX

INDEX

| | PAGE |
|--|------|
| INDEX OF AUTHORS | iii |
| GENERAL INDEX | iii |
| INDEX OF GENERA AND SPECIES NEW TO SCIENCE | viii |

INDEX OF AUTHORS

| | PAGE | | PAGE |
|---|----------------------|---|---------------|
| Bezzi, M. | 333 | Macfie, J. W. S. ; and Fraser, M. W. | 83 |
| Blacklock, B. ; and Carter, H. F. | 115, 275 | Macfie, J. W. S. ; and Ingram, A. ... | 147 |
| Blacklock, B. ; and others..... | 365, 371 | Macfie, J. W. S. ; and others | 365, 371 |
| Breinl, A. | 389 | Macfie, J. W. S. ; and Yorke 153, 159, 165, | 169, 175 |
| Carter, H. F. ; and Blacklock, B. | 115, 275 | Maplestone, P. A. | 283 |
| Carter, H. F. ; Ingram, A. ; and | | Southwell, T. | 181, 295, 355 |
| Macfie, J. W. S. | 187, 211, 309 | Southwell, T. ; and Yorke, W. | 127 |
| Carter, H. F. ; and others | 371 | Stephens, J. W. W. | 341 |
| Cooper, C. F. ; and others | 371 | Stephens, J. W. W. ; and others | 365, 371 |
| Edwards, F. W. | 23 | Swellengrebel, N. H. | 41 |
| Fraser, M. W. ; and Macfie, J. W. S. | 83 | Swellengrebel de Graaf, J. M. H. ... | 41 |
| Gaskell, J. F. | 3 | Yorke, W. ; and Macfie, J. W. S. 153, 159, | 165, 169, 175 |
| Ingram, A. ; Carter, H. F. ; and | | Yorke, W. ; and Southwell, T. | 127 |
| Macfie, J. W. S. | 187, 211, 309 | Yorke, W. ; and others | 365, 371 |
| Ingram, A. ; and Macfie, J. W. S. ... | 147 | | |
| Kotlán, A. | 299 | | |
| Macfie, J. W. S. | 73, 93, 95, 111, 137 | | |
| Macfie, J. W. S. ; Carter, H. F. ; and | | | |
| Ingram, A. | 187, 211, 309 | | |

GENERAL INDEX

| | PAGE | | PAGE |
|---|------|---|------|
| <i>Agchylostoma duodenale</i> in inhabitants | | Bahr's monograph on Filariasis | 341 |
| of Queensland | 291 | Bezzi, M. <i>Musca inferior</i> , Stein, type | |
| Albumen, excretion of, in blackwater | | of a new genus of <i>Philaematomyia</i> | |
| fever | 12 | flies | 333 |
| <i>Anopheles plumbeus</i> , Breeding-places of | 115 | Blacklock, B., and Carter, H. F. | |
| " " Further experi- | | Further experiments with <i>Anopheles</i> | |
| " " ments with..... | 275 | <i>plumbeus</i> , Stephens ; its infection | |
| " " infection with | | with <i>P. falciparum</i> in England ; also | |
| <i>P. falciparum</i> in | | notes on the apparatus and tech- | |
| England | 275 | nique employed | 275 |
| <i>Anoplocephala vulgaris</i> , sp.n. | 355 | Blacklock, B., and Carter, H. F. On | |
| Arsenic in malignant tertian malaria... | 83 | the results obtained from surveys | |
| <i>Atrichopogon</i> genus | 321 | for breeding-places of tree-hole | |
| " <i>xanthoaspidium</i> , sp.n. ... | 322 | mosquitoes in Liverpool and neigh- | |
| <i>Avitellina centripunctata</i> in an ox | 295 | bourhood | 115 |

| | PAGE |
|--|------|
| Blacklock, B., and others. Have differential leucocyte counts any value ? | 371 |
| Blacklock, B., and others. Studies in the Treatment of Malaria | 365 |
| Blackwater fever, Excretion of haemoglobin and of albumen in..... | 12 |
| " " in Macedonia..... | 3 |
| " " Quinine tolerance in | 9 |
| " " Relationship of malaria to | 5 |
| " " Relationship of quinine administration to | 7 |
| " " Seasonal incidence.. | 6 |
| " " Treatment of..... | 14 |
| <i>Blastocystis hominis</i> in inhabitants of Queensland | 291 |
| Breeding-places of tree-hole mosquitoes | 115 |
| Breinl; A. Preliminary note on the development of the larvae of <i>Dirofilaria immitis</i> in dog-fleas <i>Ctenocephalus felis</i> and <i>canis</i> | 389 |
| Cardiac aneurysms in Gold Coast..... | 147 |
| Carter, H. F., and Blacklock, B. Further experiments with <i>Anopheles plumbeus</i> , Stephens; its infection with <i>P. falciparum</i> in England; also notes on the apparatus and technique employed | 275 |
| Carter, H. F., and Blacklock, B. On the results obtained from surveys for breeding-places of tree-hole mosquitoes in Liverpool and neighbourhood | 115 |
| Carter, H. F., Ingram, A., and Macfie, J. W. S. Observations on the Ceratopogonine midges of the Gold Coast, with descriptions of new species.....187, 211, | 309 |
| Carter, H. F., and others. Have differential leucocyte counts any value ? | 371 |
| <i>Ceratopogoninae</i> of the Gold Coast 187, 211, | 309 |
| " bionomics | 195 |
| " examination technique | 192 |
| " systematic account | 211, |
| | 309 |
| Cestode, New species of, from a rhinoceros | 355 |

| | |
|---|-----|
| Cestodes in new hosts | 29 |
| Ceylon, Filariasis and elephantiasis in | 347 |
| <i>Chilomastix mesnili</i> in inhabitants of Queensland | 284 |
| Cooper, C. F., and others. Have differential leucocyte counts any value ? | 371 |
| <i>Craterostomum</i> genus | 300 |
| <i>Crossocephalus longicaudatus</i> | 135 |
| " <i>viviparus</i> | 134 |
| " <i>zebrae</i> , n.sp..... | 127 |
| <i>Ctenocephalus canis</i> , Development of larvae of <i>Dirofilaria immitis</i> in | 389 |
| " <i>felis</i> , Development of larvae of <i>Dirofilaria immitis</i> in | 389 |
| <i>Culicoides</i> genus, bionomics | 197 |
| " " morphology of adult | 212 |
| " " " larva | 224 |
| " " " pupa | 221 |
| " " synoptic tables | 267 |
| " <i>accraensis</i> , sp.n., adult | 241 |
| " " " pupa | 243 |
| " " " larva | 244 |
| " <i>austeni</i> , sp.n., adult | 261 |
| " <i>citronaeus</i> , sp.n., adult..... | 259 |
| " <i>clarkae</i> , sp.n., adult | 246 |
| " " " pupa | 248 |
| " " " larva | 249 |
| " <i>confusus</i> , sp.n., adult | 250 |
| " <i>distinctipennis</i> , adult | 238 |
| " <i>eriodendroni</i> , sp.n., adult ... | 250 |
| " " " pupa ... | 251 |
| " " " larva ... | 252 |
| " <i>fulvithorax</i> , adult | 230 |
| " <i>grabami</i> , adult | 263 |
| " <i>inornatipennis</i> , sp.n., adult... | 227 |
| " " " pupa... .. | 228 |
| " " " larva... .. | 230 |
| " <i>neavei</i> , adult | 245 |
| " <i>nigripennis</i> , sp.n., adult..... | 253 |
| " " " pupa..... | 254 |
| " " " larva ... | 254 |
| " <i>pallidipennis</i> , sp.n., adult ... | 265 |
| " <i>praetermissus</i> , sp.n., adult.... | 240 |
| " <i>punctithorax</i> , sp.n., adult ... | 235 |
| " " " pupa ... | 236 |
| " <i>schultzei</i> , adult | 231 |
| " " pupa | 234 |
| " " larva | 235 |
| " <i>similis</i> , sp.n., adult | 255 |

| | PAGE |
|--|-----------------|
| <i>Cylicostomum</i> genus | 299 |
| " <i>iblei</i> , sp.n. | 300 |
| " <i>prionodes</i> , sp.n. | 305 |
| " <i>tridentatum</i> , sp.n. | 153 |
| " <i>triramosum</i> , sp.n. | 175 |
| <i>Cylindropharynx rhodesiensis</i> , sp.n. | 169 |
| <i>Dasyhelea</i> , Bionomics of | 202 |
| <i>Davainea cesticillus</i> in guinea-fowls ... | 296 |
| " <i>tetragona</i> , in guinea-fowls ... | 296 |
| Development of larvae of <i>Dirofilaria immitis</i> in dog-fleas <i>Ctenocephalus felis</i> and <i>canis</i> | 389 |
| Differential leucocyte counts, Value of | 371 |
| Diptera Nematocera, Hypopygium of | 23 |
| <i>Dirofilaria immitis</i> , development of larvae in dog-fleas | 389 |
| Edwards, F. W. The nomenclature of the parts of the male hypopygium of Diptera Nematocera, with special reference to mosquitoes | 23 |
| Elephantiasis in Ceylon | 347 |
| " " Fiji | 343 |
| " Incidence of, among those infected with microfilaria | 343 et seq. |
| <i>Entamoeba coli</i> in inhabitants of Queensland | 284 |
| " <i>histolytica</i> in inhabitants of Queensland | 284 |
| Fiji, Filariasis and elephantiasis in..... | 341 |
| Filarial disease in Ceylon | 347 |
| " " relation to infection with microfilaria ... | 342 |
| Filariasis, Some statistics of | 341 |
| Fish and mosquito larvae | 181 |
| Fleas, Development of larvae of <i>Dirofilaria immitis</i> in | 389 |
| Fraser, M. W., and Macfie, J. W. S. Oral administration of quinine or quinine and arsenic for short periods to young native children infected with malignant tertian malaria | 83 |
| Gaskell, J. F. Notes on blackwater fever in Macedonia | 3 |
| <i>Giardia intestinalis</i> in inhabitants of Queensland | 284 |
| Gold Coast, cardiac aneurysms in native boys in..... | 147 |
| " " Ceratopogonine midges of | 187, 211, 309 |
| " " Treatment of malaria in | 83, 93, 95, 111 |

| | PAGE |
|--|---------------|
| Guinea-worm infections, Tartar emetic in | 137 |
| Haemoglobin, Excretion of, in black-water fever | 12 |
| <i>Haplochilus</i> , genus of larvae-eating fish | 183 |
| Horses, <i>Strongylidae</i> in, 153, 159, 165, 169, 175 | |
| Human intestinal protozoa in North Queensland | 283 |
| <i>Hymenolepis</i> sp. in inhabitants of Queensland | 291 |
| Hypopygium, male, of Diptera Nematocera | 23 |
| 'I' cysts in inhabitants of Queensland | 290 |
| India, Fish and mosquito larvae in ... | 181 |
| Ingram, A., Carter, H. F., and Macfie, J. W. S. Observations on the Ceratopogonine midges of the Gold Coast, with descriptions of new species..... | 187, 211, 309 |
| Ingram, A., and Macfie, J. W. S. Three cases of cardiac aneurysm in native boys of the Gold Coast | 147 |
| Intestinal protozoa in North Queensland | 283 |
| " protozoal infection, Age incidence in | 286 |
| " " infection, Sex incidence in | 287 |
| Jamaica, <i>Strongylidae</i> in | 165 |
| Kotlán, A. Two new <i>Cylicostomum</i> species from the horse | 299 |
| <i>Iamblia intestinalis</i> in inhabitants of Queensland | 284 |
| Larvae-eating fish | 183 |
| Leucocyte counts, differential, Value of | 371 |
| Macfie, J. W. S. Heat and <i>Stegomyia fasciata</i> | 73 |
| Macfie, J. W. S. Oral administration of quinine sulphate grains 20 to adult natives infected with malignant tertian malaria | 93 |
| Macfie, J. W. S. Oral administration of quinine sulphate grains 10 daily for two consecutive days only to native school-boys infected with malignant tertian malaria | 95 |
| Macfie, J. W. S. Oral administration of quinine sulphate to natives infected with quartan and simple tertian malaria | 111 |

| | PAGE |
|---|---------------|
| Macfie, J. W. S. Tartar emetic in guinea-worm infections | 137 |
| Macfie, J. W. S., Carter, H. F., and Ingram, A. Observations on the Ceratopogonine midges of the Gold Coast, with descriptions of new species..... | 187, 211, 309 |
| Macfie, J. W. S., and Fraser, M. W. Oral administration of quinine or quinine and arsenic for short periods to young native children infected with malignant tertian malaria..... | 83 |
| Macfie, J. W. S., and Ingram, A. Three cases of cardiac aneurysm in native boys of the Gold Coast | 147 |
| Macfie, J. W. S., and others. Have differential leucocyte counts any value? | 371 |
| Macfie, J. W. S., and others. Studies in the Treatment of Malaria | 365 |
| Macfie, J. W. S., and Yorke, W. <i>Strongylidae</i> in Horses — | |
| IX. <i>Cylicostomum tridentatum</i> , sp.n. | 153 |
| X. On the genus <i>Poteriostomum</i> , Quiel | 159 |
| XI. Species found in West Africa and Jamaica | 165 |
| XII. <i>Cylindropharynx rhodesiensis</i> , sp.n. | 169 |
| XIII. <i>Cylicostomum triramorum</i> , sp.n. | 175 |
| Malaria in Malay, endemic type | 43 |
| „ „ epidemic type | 48 |
| „ „ sub-endemic type... .. | 46 |
| „ malignant tertian, Treatment of | 83, 93, 95 |
| „ quartan, Treatment of | 111 |
| „ relationship to blackwater fever .. | 5 |
| „ simple tertian, Time of onset of paroxysms in | 365 |
| „ „ „ Treatment of | 111 |
| Malay Archipelago, Malaria in | 41 |
| Maplestone, P. A. Human intestinal protozoa in North Queensland | 283 |
| <i>Metroliasthes lucida</i> in guinea-fowls ... | 296 |
| Microfilaria, Infection with, and relation to filarial disease | 342 |
| Midges of the Gold Coast | 187, 211, 309 |
| <i>Moniezia expansa</i> , in a duiker | 296 |
| „ <i>oblongiceps</i> in a water-buck | 296 |
| Mosquitoes, Tree-hole breeding places of, in Liverpool district | 115 |

| | PAGE |
|--|---------------|
| <i>Musca crassirostris</i> | 337 |
| „ <i>indica</i> | 338 |
| „ <i>inferior</i> , Specific description of .. | 335 |
| „ „ type of a new genus of <i>Philaematomyia</i> flies | 333 |
| <i>Myzomyia ludlowi</i> , carrier of malaria in Malay | 43 |
| Nematode, New species of, in horses .. | 153, 169, 175 |
| „ „ in a zebra | 127 |
| Nomenclature of the parts of the male hypopygium of Diptera Nematocera .. | 23 |
| Note on the occurrence of certain cestodes in new hosts | 295 |
| Observations on the Ceratopogonine Midges of the Gold Coast with descriptions of new species | 187, 211, 309 |
| <i>Ochlerotatus geniculatus</i> , Breeding-places of | 115 |
| <i>Oxyuris vermicularis</i> in inhabitants of Queensland | 291 |
| Paroxysms in simple tertian malaria, Time of onset of | 365 |
| <i>Philaematomyia crassirostris</i> | 337 |
| „ <i>gurnei</i> | 337 |
| „ <i>indica</i> | 338 |
| „ <i>inferior</i> | 337 |
| „ <i>insignis</i> | 337 |
| „ <i>lineata</i> | 338 |
| <i>Plasmodium falciparum</i> , infecting <i>A. plumbeus</i> in England ... | 275 |
| „ <i>vixax</i> , infecting <i>A. plumbeus</i> in England | 275 |
| <i>Poteriostomum</i> genus | 159 |
| „ „ variety of <i>C. ratzii</i> | 299 |
| <i>Prionognathus</i> , gen. n. | 309 |
| „ <i>maculipennis</i> , sp.n. | 316 |
| „ <i>maculithorax</i> , sp.n. ... | 319 |
| „ <i>marmoratus</i> , sp.n. | 312 |
| „ <i>pseudomaculipennis</i> , sp.n. | 318 |
| <i>Pristirrhynchomyia indica</i> | 338 |
| „ <i>lineata</i> | 338 |
| <i>Ptilolepis</i> , gen. n. | 335 |
| „ <i>inferior</i> | 337 |
| Protozoa, intestinal, in North Queensland | 283 |
| Queensland, Human intestinal protozoa in..... | 283 |
| Quinine administration, Relationship of, to blackwater fever..... | 7 |
| „ in malignant tertian malaria | 83, 93, 95 |

| | PAGE |
|--|------|
| Quinine in quartan malaria | 111 |
| „ in simple tertian malaria | 111 |
| „ tolerance in blackwater fever | 9 |
| „ treatment in malaria in Malay | 48 |
| Results obtained from surveys for breeding-places of tree-hole mosquitoes in Liverpool and neighbourhood | 115 |
| Rhinoceros, New species of cestode from | 355 |
| Southwell, T. Fish and mosquito larvae in Bengal, Bihar, and Orissa, India | 181 |
| Southwell, T. A new species of cestode (<i>Anoplocephala vulgaris</i>) from an African rhinoceros | 355 |
| Southwell, T. A note on the occurrence of certain cestodes in new hosts | 295 |
| Southwell, T., and Yorke, W. <i>Crossocephalus zebrae</i> , n.sp. | 127 |
| Statistics of filariasis | 341 |
| <i>Stegomyia fasciata</i> , short exposures to raised temperatures | 73. |
| Stephens, J. W. W. Some statistics of filariasis | 341 |
| Stephens, J. W. W., and others. Have differential leucocyte counts any value ? | 371 |
| Stephens, J. W. W., and others. Studies in the Treatment of Malaria | 365 |
| <i>Stilesia globipunctata</i> in a water-buck... .. | 296 |
| „ <i>hepatica</i> in cattle | 295 |
| <i>Stilobezzia</i> genus | 324 |
| „ <i>spirogyrae</i> , sp.n., adult..... | 325 |
| „ „ „ pupa ... | 328 |
| „ „ „ larva ... | 330 |
| <i>Strongylidae</i> in Horses, 153, 159, 165, 169, | 175 |
| <i>Strongyloides stercoralis</i> in inhabitants of Queensland | 291 |

| | PAGE |
|--|------|
| Studies in the Treatment of Malaria— | |
| XXXI. The time of onset of the paroxysms in simple tertian malaria | 365 |
| Swellengrebel (N. H.) and Swellengrebel de Graaf (J. M. H.). Studies on the various types of malarial infection and the effect of quinine treatment thereon among the native population of the Malay Archipelago | 41 |
| <i>Taenia</i> ? <i>saginata</i> in a goat | 297 |
| Tartar emetic in guinea-worm infections | 137 |
| <i>Tetramitus mesnili</i> in inhabitants of Queensland | 284 |
| Time of onset of paroxysms in simple tertian malaria | 365 |
| <i>Trichuris trichiura</i> in inhabitants of Queensland | 291 |
| Two new <i>Cylicostomum</i> species from the horse | 299 |
| Urine, Suppression of, in blackwater fever | 13 |
| West Africa, <i>Strongylidae</i> in | 165 |
| Yorke, W., and Macfie, J. W. S. <i>Strongylidae</i> in Horses :— | |
| IX. <i>Cylicostomum tridentatum</i> , sp.n. | 153 |
| X. On the genus <i>Poteriostomum</i> , Quiel | 159 |
| XI. Species found in West Africa and Jamaica | 165 |
| XII. <i>Cylindropharynx rhodesiensis</i> , sp.n. | 169 |
| XIII. <i>Cylicostomum triramosum</i> , sp.n. | 175 |
| Yorke, W., and others. Have differential leucocyte counts any value ? ... | 371 |
| Yorke, W., and others. Studies in the Treatment of Malaria | 365 |
| Yorke, W., and Southwell, T. <i>Crossocephalus zebrae</i> , n.sp. | 127 |

INDEX OF GENERA AND SPECIES NEW TO SCIENCE

| | PAGE | | PAGE |
|--|------|---|------|
| <i>Anoplocephala vulgaris</i> ... | 355 | <i>Culicoides similis</i> ... | 255 |
| <i>Atrichopogon xanthoaspidium</i> ... | 322 | <i>Cylicostomum iblei</i> ... | 300 |
| <i>Crossocephalus zebrae</i> ... | 127 | <i>Cylicostomum prionodes</i> ... | 305 |
| <i>Culicoides accraensis</i> ... | 241 | <i>Cylicostomum tridentatum</i> ... | 153 |
| <i>Culicoides austeni</i> ... | 261 | <i>Cylicostomum triramosum</i> ... | 175 |
| <i>Culicoides citroneus</i> .. | 259 | <i>Cylindropharynx rhodesiensis</i> ... | 169 |
| <i>Culicoides clarkei</i> ... | 246 | <i>Prionognathus</i> gen. ... | 309 |
| <i>Culicoides confusus</i> ... | 250 | <i>Prionognathus maculipennis</i> ... | 316 |
| <i>Culicoides eriodendroni</i> ... | 250 | <i>Prionognathus maculithorax</i> ... | 319 |
| <i>Culicoides inornatipennis</i> ... | 227 | <i>Prionognathus marmoratus</i> ... | 312 |
| <i>Culicoides nigripennis</i> ... | 253 | <i>Prionognathus pseudomaculipennis</i> ... | 318 |
| <i>Culicoides pallidipennis</i> ... | 265 | <i>Ptilolepis</i> gen. ... | 335 |
| <i>Culicoides praetermissus</i> ... | 240 | <i>Stilobezzia spirogyrae</i> ... | 325 |
| <i>Culicoides punctithorax</i> ... | 235 | | |

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